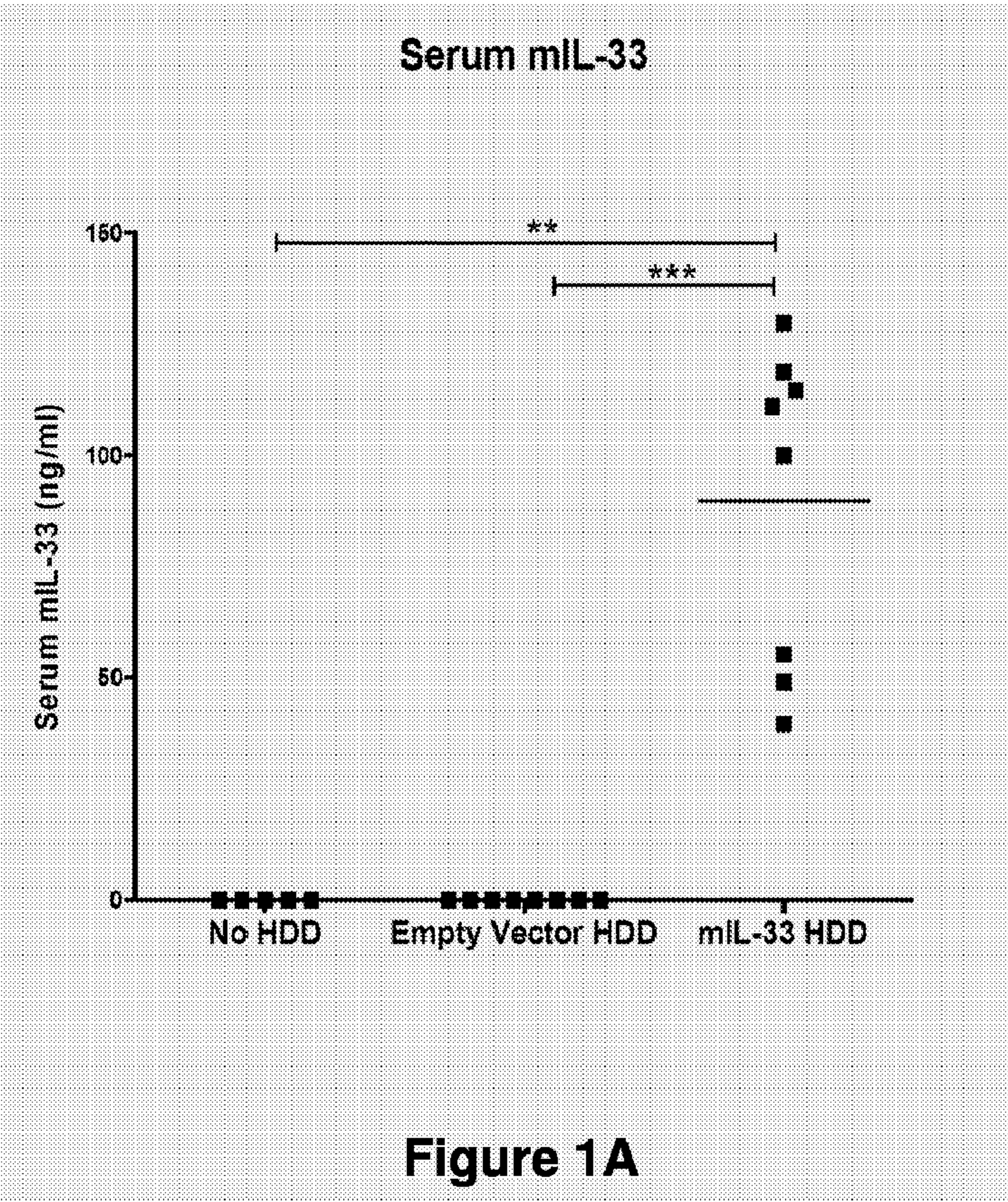




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(54) **Titre : BIOMARQUEURS ASSOCIES AUX MALADIES MEDIEES PAR L'INTERLEUKINE-33 (IL-33) ET LEURS UTILISATIONS**
(54) **Title: BIOMARKERS RELATED TO INTERLEUKIN-33 (IL-33)-MEDIATED DISEASES AND USES THEREOF**



(57) **Abrégé/Abstract:**
The present invention relates to the identification of certain biomarkers for use in identifying patients who have, or are likely to develop an IL-33 mediated disease or disorder and who are more likely to respond to therapy with an IL-33 antagonist. The

(57) Abrégé(suite)/Abstract(continued):

invention also relates to methods of treatment of an IL-33-mediated disease or disorder in a patient by administering an IL-33 antagonist to the patient in need thereof and monitoring the effectiveness of therapy using the biomarkers described herein. Also provided are methods for decreasing the level of at least one biomarker in a subject suffering from an IL-33-mediated disease or disorder, and methods for treating such diseases or disorders according to the expression levels of one or more biomarkers. The methods of the present invention comprise administering to a subject in need thereof a pharmaceutical composition comprising an interleukin-33 antagonist.

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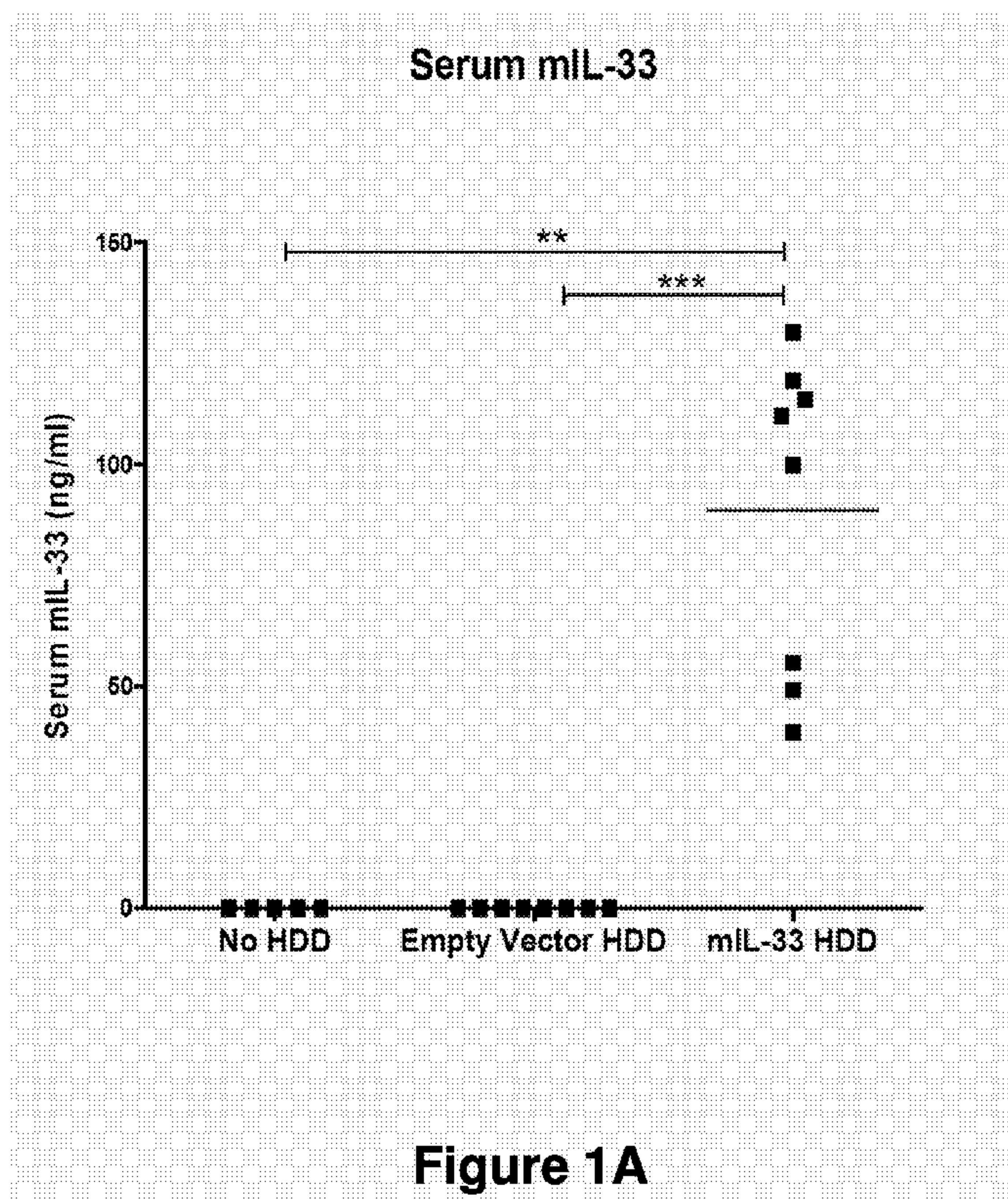
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(54) Title: BIOMARKERS RELATED TO INTERLEUKIN-33 (IL-33)-MEDIATED DISEASES AND USES THEREOF



(57) Abstract: The present invention relates to the identification of certain biomarkers for use in identifying patients who have, or are likely to develop an IL-33 mediated disease or disorder and who are more likely to respond to therapy with an IL-33 antagonist. The invention also relates to methods of treatment of an IL-33-mediated disease or disorder in a patient by administering an IL-33 antagonist to the patient in need thereof and monitoring the effectiveness of therapy using the biomarkers described herein. Also provided are methods for decreasing the level of at least one biomarker in a subject suffering from an IL-33-mediated disease or disorder, and methods for treating such diseases or disorders according to the expression levels of one or more biomarkers. The methods of the present invention comprise administering to a subject in need thereof a pharmaceutical composition comprising an interleukin-33 antagonist.

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BIOMARKERS RELATED TO INTERLEUKIN-33 (IL-33)-MEDIATED DISEASES AND USES THEREOF

REFERENCE TO A SEQUENCE LISTING

[0001] This application incorporates by reference the Sequence Listing submitted in Computer Readable Form as file 10187WO01-Sequence.txt, created on October 4, 2016 and containing 159,920 bytes.

FIELD OF THE INVENTION

[0002] The present invention relates to the identification of particular biomarkers in a mammal that correlate with expression of IL-33 and the use of these biomarkers to identify patients who have, or are likely to develop, an IL-33 mediated disease or disorder and who are more likely to respond to therapy with an IL-33 antagonist. The invention also relates to methods of treatment of an IL-33-mediated disease or disorder in a patient by administering an IL-33 antagonist to the patient in need thereof and monitoring the effectiveness of therapy using the biomarkers described herein.

BACKGROUND

[0003] Interleukin-33 (IL-33), which is a member of the IL-1 superfamily of cytokines, is expressed predominantly by stromal cells, such as epithelial and endothelial cells, following pro-inflammatory stimulation. IL-33 is a ligand for ST2, a toll-like/interleukin-1 receptor super-family member that associates with an accessory protein, IL-1RAcP (for reviews, see, e.g., Kakkar and Lee, *Nature Reviews – Drug Discovery* 7(10):827-840 (2008), Schmitz et al., *Immunity* 23:479-490 (2005); Liew et al., *Nature Reviews – Immunology* 10:103-110 (2010); US 2010/0260770; US 2009/0041718). Upon activation of ST2/IL-1RAcP by IL-33, a signaling cascade is triggered through downstream molecules such as MyD88 (myeloid differentiation factor 88) and TRAF6 (TNF receptor associated factor 6), leading to activation of NFkB (nuclear factor-κB), among others.

[0004] IL-33 signaling has been implicated as a factor in a variety of diseases and disorders (Liew et al., *Nature Reviews – Immunology* 10:103-110 (2010)). For example, while IL-33 is protective against helminth infection in a host and also reduces atherosclerosis by promoting T_H2-type immune responses, it can also promote the pathogenesis of asthma by expanding T_H2 cells and mediate joint inflammation, atopic dermatitis and anaphylaxis by mast cell activation. As such, IL-33 may be a new target for therapeutic intervention across a range of diseases; for example, blockade of IL-33 signaling may offer the potential to ameliorate multiple pathogenic features of certain inflammatory diseases.

[0005] IL-33 antagonists are described in U.S. patent numbers 5,576,191; 7,666,622; 8,119,771; 8,187,596; 9,090,694; 9,212,227; 9,382,318; U.S. patent publication numbers 2007/0042978; 2009/0041718; 2010/0260770; 2012/0207752; 2012/0263709; 2014/0140954;

2014/0271642; 2014/0212412; EP patents or patent application numbers 1725261B1; 2069784A1; 2152740A1; 2283860A2; 2475388A1; and PCT publication numbers WO05/079844; WO08/132709; WO08/144610; WO09/053098; WO11/031600; WO14/152195 and WO14/164959.

[0006] Calcitonin (CT) is a polypeptide hormone, thought to be produced primarily by parafollicular cells of the thyroid (Foster, G. V., *et al.*, (1964), *Nature* 202: 1303-1305). CT is a product of the *CALCA* gene, located on chromosome 11. The *CALCA* gene encodes the polypeptides procalcitonin (PCT) and PCT gene-related peptide α (proCGRP α), which are differentially expressed by alternative splicing (Christ-Crain, M. *et al.* (2008), *Crit Care Med* 36:1684-1687; Hoff, AO, *et al.* (2002), *J. Clin. Invest.* 110:1849-1857). Calcitonin (CT) is derived from a larger precursor, Procalcitonin (PCT, 116 amino acids), which is cleaved to immature calcitonin (33 amino acids) and then to mature calcitonin, a monomer of a 3.5-kd peptide composed of 32 amino acids. CT is predominantly produced by neuroendocrine cells such as C cells of the thyroid and pulmonary neuroendocrine cells (PNECs) by proteolytic cleavage in the secretory granules of producing cells before being released as mature CT polypeptide. Other cells that express the *calca* gene include mast cells, dorsal root ganglion cells (DRGs) and cells of the spinal cord. Multiple forms of circulating calcitonin precursors are found in the serum of healthy and diseased individuals (Becker K. *et al.* (2004), *J Clin Endocrinol Metab* 89:1512-1525).

[0007] Calcitonin gene related peptide (CGRP) is a member of the calcitonin family of peptides. α -CGRP is a 37-amino acid peptide formed from the alternative splicing of the calcitonin/CGRP gene located on chromosome 11 (Amara, SG, *et al.*, (1982), *Nature*, 298 (5871): 240-244).

[0008] CT acts to reduce blood calcium, inhibits both osteoclasts and bone resorption, opposing the activity of parathyroid hormone (PTH) (Naot, D. and J. Cornish (2008), *Bone* 43(5): 813-818). *In vivo* and *in vitro* experiments suggest that the major physiological function of CT is to combat hypercalcemia in states of calcium stress such as during pregnancy, growth or lactation (Hoff, A. O., *et al.*, (2002), *J Clin Invest* 110(12): 1849-1857; Zaidi, M., *et al.*, (2002), *J Clin Invest* 110(12): 1769-1771). Diagnostically, CT is used as biomarker for medullary thyroid carcinoma (MTC). Normal circulating levels of CT peptides are low, however under physiological conditions these levels increase either systemically or locally. High CT levels indicate the presence of MTC and are used to evaluate the efficacy of surgical extirpation and recurrences. CT is also elevated in C-cell hyperplasia, pulmonary and pancreatic tumors, kidney failures and thyroid autoimmune disease (Becker, K. L., *et al.*, (2004). *J Clin Endocrinol Metab* 89(4): 1512-1525).

[0009] To date, no biomarkers for identifying patients that have, or are prone to develop an IL-33 mediated disease or disorder, or to determine a patient's likelihood to respond to therapy with an IL-33 antagonist have been identified. Although IL-33 antagonists have been identified

that show promise in the treatment of inflammatory conditions, or allergies, biomarkers that may predict the efficacy of anti-IL-33 therapy are needed for the effective identification and selection of patient sub-populations that respond favorably to anti-IL-33 therapy. Accordingly, an unmet need exists in the art for identifying and validating predictive and prognostic biomarkers in patients with inflammatory conditions, or allergies, who are administered anti-IL-33 therapy.

BRIEF SUMMARY OF THE INVENTION

[0010] According to certain aspects of the present invention, methods are provided for treating, preventing and/or reducing the severity or duration of symptoms of an interleukin-33 (IL-33)-mediated disease or disorder in a subject. The methods of the present invention comprise administering to a subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of an interleukin-33 (IL-33) antagonist.

[0011] In one embodiment of the present invention, the IL-33 antagonist is an antibody or antigen-binding fragment thereof that specifically binds IL-33.

[0012] In one embodiment of the present invention, the IL-33 antagonist is a receptor-based antagonist of IL-33, such as an IL-33 trap, as described herein.

[0013] The present invention also provides for the identification of particular biomarkers that correlate with the expression of IL-33 in a subject suffering from an IL-33 mediated disease or disorder. Exemplary biomarkers associated with an IL-33-mediated disease or disorder that can be evaluated and/or measured in the context of the present invention include, but are not limited to, polypeptides encoded by the *CALCA* gene, including calcitonin, procalcitonin, and calcitonin gene-related peptide (CGRP). In one embodiment, the biomarker associated with an IL-33-mediated disease or disorder that can be evaluated and/or measured in the context of the present invention is calcitonin. Other biomarkers associated with an IL-33-mediated disease or disorder that can be evaluated and/or measured in the context of the present invention include, but are not limited to resistin-like alpha (RETNA); chemokine (C-C motif) ligand 8 (Ccl8); serum amyloid A 3 (Saa3); Gm1975 (BC117090); killer cell lectin-like receptor (Klrk1); stefin A1 (Csta); membrane-spanning 4-domain (Ms4a8a); chemokine (C-C motif) ligand 11 (Ccl11); and serine (or cysteine) peptides (Serpina3f).

[0014] In a related aspect, the invention provides for the expression of at least one biomarker in the serum of a subject suffering from an IL-33 mediated disease or disorder, which correlates with the level of expression of IL-33 in at least one tissue sample from the subject.

[0015] In one embodiment, the serum biomarker that correlates with IL-33 expression levels in at least one tissue sample is calcitonin.

[0016] In a certain embodiment, the serum biomarker that correlates with IL-33 expression levels in at least one tissue sample is procalcitonin.

[0017] In a related embodiment, the serum biomarker that correlates with IL-33 expression levels in at least one tissue sample is calcitonin gene-related peptide (CGRP).

[0018] In another related aspect, the present invention provides a method for treating an interleukin-33 (IL-33) mediated disease or disorder in a subject, the method comprising: (a) selecting a subject who exhibits an elevated level of at least one biomarker associated with an IL-33 mediated disease or disorder prior to, or at the time of treatment, and (b) administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of an interleukin-33 antagonist.

[0019] According to a related aspect of the present invention, methods for treating an IL-33-mediated disease or disorder are provided which comprise administering to a subject a pharmaceutical composition comprising a therapeutically effective amount of an IL-33 antagonist, wherein administration of the pharmaceutical composition to the subject results in a decrease in at least one biomarker associated with an IL-33-mediated disease or disorder in the subject.

[0020] In one embodiment, the biomarker that is elevated in a subject suffering from an IL-33 mediated disease or disorder is calcitonin and the IL-33 antagonist is a monoclonal antibody that specifically binds to IL-33.

[0021] The present invention also provides methods for decreasing the level of one or more IL-33-mediated disease or disorder-associated biomarker(s) in a subject, which results in improving one or more IL-33-mediated disease or disorder-associated parameter(s) in a subject, wherein the methods comprise administering to a subject in need thereof a single initial dose of a pharmaceutical composition comprising an IL-33 antagonist. In certain embodiments, the invention provides administering one or more secondary doses of the pharmaceutical composition comprising the IL-33 antagonist, wherein the administering results in further reduction in the level of one or more of the biomarkers described herein and further improvement in one or more IL-33 mediated disease or disorder-associated parameters in a subject. In one embodiment, there is a strong correlation between the level of the biomarker present in the serum of the subject having an IL-33 mediated disease or disorder and the severity of the disease or disorder-associated parameter, e.g. infiltration of eosinophils or neutrophils into the lungs of the subject, or an increase in cytokine levels (e.g. IL-5) in the lungs of the subject. In certain embodiments, the level of IL-33 in the tissue, e.g. the lung, correlates with the severity of the disease or disorder-associated parameter. In one embodiment, the IL-33 antagonist is a human monoclonal antibody that binds specifically to IL-33. In one embodiment, the IL-33 antagonist is an IL-33 trap, as described herein.

[0022] In a related aspect, the present invention provides methods for treating an IL-33-mediated disease or disorder in a subject comprising administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of an antibody or antigen-binding fragment thereof that specifically binds IL-33, wherein the subject has been diagnosed with an IL-33-mediated disease or disorder and has also been selected for treatment on the basis of the subject exhibiting an elevated level of at least one IL-33-mediated disease or

disorder-associated biomarker before treatment, as compared to a reference level of the biomarker (*e.g.*, expression of the biomarker in a subset of subjects diagnosed with an IL-33-mediated disease or disorder and/or expression of the biomarker in healthy subjects).

[0023] In another related aspect, the present invention also provides methods for treating an IL-33-mediated disease or disorder by administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of an antibody or antigen-binding fragment thereof that specifically binds IL-33, wherein the subject has been diagnosed with an IL-33-mediated disease or disorder, has already been treated with the anti-IL33 antagonist for a defined period of time, and has been selected for further treatment with the IL-33 antagonist on the basis of exhibiting expression of at least one biomarker (*e.g.*, calcitonin, procalcitonin, or calcitonin gene-related peptide (CGRP)) after treatment for a defined period of time, wherein the expression of the biomarker is determined based on a comparison to the level of expression of the respective biomarker in the subject prior to treatment with the anti-IL-33 antagonist.

[0024] The invention also provides a method for identifying a subject suffering from an IL-33 mediated disease or disorder who is likely to respond favorably to therapy with an IL-33 antagonist, the method comprising obtaining a sample from the subject and measuring the level of calcitonin in the sample; wherein an elevated level of calcitonin, as compared to the lower level of calcitonin in subjects not suffering from an IL-33 mediated disease or disorder, identifies the patient as a patient who is likely to respond favorably to therapy with an IL-33 antagonist.

[0025] In certain embodiments, the subject who is likely to respond favorably to therapy with an IL-33 antagonist may be identified by measuring the level of procalcitonin, or calcitonin gene-related peptide (CGRP) in a tissue sample, wherein an elevated level of either procalcitonin, or calcitonin gene-related peptide (CGRP), as compared to the lower level of procalcitonin, or CGRP in subjects not suffering from an IL-33 mediated disease or disorder, identifies that patient as a patient who is likely to respond favorably to therapy with an IL-33 antagonist.

[0026] In another related aspect, the invention provides a method of determining whether a subject is likely to have, or likely to develop an IL-33 mediated disease or disorder, the method comprising obtaining a sample from the subject and measuring in the sample the level of calcitonin; wherein an elevated level of calcitonin, as compared to a reference standard, identifies the patient as a patient who is likely to have, or develop an IL-33 mediated disease or disorder.

[0027] In certain embodiments, the invention provides a method of determining whether a subject is likely to have, or likely to develop an IL-33 mediated disease or disorder, the method comprising obtaining a sample from the subject and measuring in the sample the level of procalcitonin, or CGRP; wherein an elevated level of procalcitonin, or CGRP, as compared to reference standards for either of the two biomarkers, identifies the patient as a patient who is likely to have, or develop an IL-33 mediated disease or disorder. Methods for measuring the levels of any of the above biomarkers are known to those of skill in the art.

[0028] The sample from the subject may be a solid tissue sample, a cell sample, or a blood sample. The solid tissue sample may be selected from the group consisting of heart, kidney, liver, lung, colon and spleen. The cell sample may be a dorsal root ganglion cell sample, sputum cell sample, bronchoalveolar lavage cell sample, nasal polyps cell sample, fecal cell sample, lung, colon heart, kidney, skin biopsy, or spleen biopsy cell sample. The blood sample may be whole blood, plasma, or serum.

[0029] In one embodiment, the IL-33 antagonist to be used in the methods of the invention is an isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds human interleukin-33 (IL-33), wherein the antibody or antigen-binding fragment comprises: (a) the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, and 308; and (b) the CDRs of a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, and 316.

[0030] In one embodiment, the IL-33 antagonist to be used in the methods of the invention is an isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds human interleukin-33 (IL-33), wherein the anti-IL-33 antibody or antigen-binding fragment thereof comprises the heavy and light chain CDRs of a HCVR/LCVR amino acid sequence pair selected from the group consisting of: SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282, 290/298, and 308/316.

[0031] In one embodiment, the IL-33 antagonist to be used in the methods of the invention is an isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds human interleukin-33 (IL-33), wherein the antibody or antigen-binding fragment comprises HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively, selected from the group consisting of: SEQ ID NOs: 4-6-8-12-14-16; 20-22-24-28-30-32; 36-38-40-44-46-48; 52-54-56-60-62-64; 68-70-72-76-78-80; 84-86-88-92-94-96; 100-102-104-108-110-112; 116-118-120-124-126-128; 132-134-136-140-142-144; 148-150-152-156-158-160; 164-166-168-172-174-176; 180-182-184-188-190-192; 196-198-200-204-206-208; 212-214-216-220-222-224; 228-230-232-236-238-240; 244-246-248-252-254-256; 260-262-264-268-270-272; 276-278-280-284-286-288; 292-294-296-300-302-304; and 310-312-314-318-320-322.

[0032] In one embodiment, the IL-33 antagonist to be used in the methods of the invention is an isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds human interleukin-33 (IL-33), wherein the antibody or antigen-binding fragment comprises: (a) a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, and 308; and (b) a light chain variable region (LCVR) having

an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, and 316.

[0033] In one embodiment, the IL-33 antagonist to be used in the methods of the invention is an isolated IL-33 receptor based antagonist (*e.g.* an IL-33 trap), comprising a first IL-33 binding domain (D1) attached to a multimerizing domain (M), wherein D1 comprises an IL-33-binding portion of an ST2 protein. In one embodiment, the IL-33 trap further comprises one or more additional IL-33 binding domains selected from the group consisting of D2, D3 and D4.

[0034] In one embodiment, the IL-33 receptor based antagonist comprises an IL-33-binding portion of an ST2 protein, an extracellular domain of an IL-1 RAcP protein, or other IL-33 binding domain.

[0035] In one embodiment, the IL-33 receptor based antagonist is an IL-33 trap comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 323, 324, 325, 326 and 335.

[0036] In one embodiment, the methods of the invention provide administration of an effective amount of a second therapeutic agent useful for diminishing at least one symptom of an IL-33 mediated disease or disorder.

[0037] In one embodiment, the second therapeutic agent is selected from the group consisting of a non-steroidal anti-inflammatory (NSAID), a corticosteroid, a bronchial dilator, an antihistamine, epinephrine, a decongestant, a thymic stromal lymphopoietin (TSLP) antagonist, an IL-13 antagonist, an IL-4 antagonist, an IL-4/IL-13 dual antagonist, an IL-5 antagonist, an IL-6 antagonist, an IL-12/23 antagonist, an IL-22 antagonist, an IL-25 antagonist, an IL-17 antagonist, an IL-31 antagonist, an oral PDE4 inhibitor and another IL-33 antagonist or a different antibody or receptor based antagonist to IL-33.

[0038] In one embodiment, the invention provides for treatment of a subject suffering from an IL-33 mediated disease or disorder with an IL-33 antagonist selected from an anti-IL-33 antibody or an IL-33 trap, which may be used in combination with any one or more of the second therapeutic agents noted above, wherein the effectiveness of treatment with these agents may be monitored by assessing the level of at least one biomarker selected from the group consisting of calcitonin (Calca), procalcitonin, calcitonin gene-related peptide (CGRP), resistin-like alpha (RETNA), chemokine (C-C motif) ligand 8 (Ccl8), serum amyloid A 3 (Saa3), Gm1975 (BC117090), killer cell lectin-like receptor (Klrk1), stefin A1 (Csta), membrane-spanning 4-domain (Ms4a8a), chemokine (C-C motif) ligand 11 (Ccl11), and serine (or cysteine) peptides (Serpina3f).

[0039] In one embodiment, the biomarker is calcitonin and the calcitonin level increases in the serum of the subject having an IL-33 mediated disease or disorder, and the increase in serum calcitonin correlates with an increased level of calcitonin and IL-33 in the lungs of the subject.

[0040] In one embodiment, the increased level of calcitonin in the serum of the subject having an IL-33 mediated disease or disorder is reduced to a reference range level following treatment

with an anti-IL-33 antagonist.

[0041] In one embodiment, a subject suffering from an IL-33 mediated disease or disorder is selected for treatment with an IL-33 antagonist on the basis of exhibiting a mean serum calcitonin level of greater than about 5 pg/ml, greater than about 10 pg/ml, greater than about 50 pg/ml, or greater than about 100 pg/ml prior to, or at the time of treatment.

[0042] In one embodiment, a subject suffering from an IL-33 mediated disease or disorder is selected for treatment with an IL-33 antagonist on the basis of exhibiting a mean serum procalcitonin level of greater than about 0.15 ng/ml, greater than about 1.0 ng/ml, greater than about 5 ng/ml, greater than about 10 ng/ml, greater than about 50 ng/ml, or greater than about 100 ng/ml prior to, or at the time of treatment.

[0043] In one embodiment, a subject suffering from an IL-33 mediated disease or disorder is selected for treatment with an IL-33 antagonist on the basis of exhibiting a mean serum calcitonin gene-related peptide (CGRP) level of greater than about 45 pg/ml, greater than about 100 pg/ml, greater than about 250 pg/ml, or greater than about 500 pg/ml prior to, or at the time of treatment.

[0044] In certain embodiments, the methods of the invention provide for treatment of an IL-33 mediated disease or disorder, which is an inflammatory disease or disorder selected from the group consisting of asthma, allergy, allergic rhinitis, allergic airway inflammation, atopic dermatitis (AD), chronic obstructive pulmonary disease (COPD), inflammatory bowel disease (IBD), multiple sclerosis, arthritis, psoriasis, eosinophilic esophagitis, eosinophilic pneumonia, eosinophilic psoriasis, hypereosinophilic syndrome, graft-versus-host disease, uveitis, cardiovascular disease, pain, multiple sclerosis, lupus, vasculitis, chronic idiopathic urticaria and Eosinophilic Granulomatosis with Polyangiitis (Churg-Strauss Syndrome).

[0045] In one embodiment, the IL-33 mediated disease or disorder to be treated is an allergy and the subject selected for treatment with an IL-33 antagonist is selected on the basis of an increase in calcitonin levels that are above normal (e.g. based on a reference level of the biomarker as determined by established values for healthy subjects compared to subjects diagnosed with an IL-33 mediated disease or disorder), wherein the treatment with an IL-33 antagonist comprises administration of a pharmaceutical composition comprising a therapeutically effective amount of a monoclonal antibody that specifically binds IL-33 and comprises an HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 274/282, and wherein the administration results in an improvement in at least one symptom associated with the IL-33 disease or disorder, or an improvement in at least one disease associated parameter; and wherein the calcitonin level is restored to a normal range as determined by comparison to a reference standard.

[0046] In one embodiment, the IL-33 mediated disease or disorder to be treated is an allergy and the subject selected for treatment with an IL-33 antagonist is selected on the basis of an increase in serum calcitonin levels as determined by comparison with a reference standard,

wherein the calcitonin level correlates with an increase in IL-33 level in the subject, or with an increase in at least one disease parameter in the subject, and wherein the increase in IL-33 level is determined using a binding assay comprising an antibody that binds specifically to human IL-33 and comprises a HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 274/282.

[0047] The present invention also includes uses of the IL-33 antagonists (*e.g.*, IL-33 trap), anti-IL-33 antibodies and pharmaceutical compositions discussed above or herein for treating, or reducing a symptom of, an IL-33 mediated disease or disorder. The present invention further includes uses of the IL-33 antagonists (*e.g.*, IL-33 trap), anti-IL-33 antibodies and pharmaceutical compositions discussed above or herein in the manufacture of a medicament for treating, or reducing a symptom of, an IL-33 mediated disease or disorder.

[0048] The present invention also includes any combination of the embodiments discussed above or herein. Particular embodiments of the present invention will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURES

[0049] Figures 1A and 1B: Show mouse serum IL-33 (Fig. 1A) and mouse Calcitonin (CT) levels (Fig. 1B) in a mouse IL-33 HDD experiment.

[0050] Figure 2: Shows mouse Calcitonin (Calca) gene expression in the chronic HDM model.

[0051] Figure 3: Shows the correlation between serum and lung Calcitonin (CT) levels in the chronic HDM model.

[0052] Figure 4: Shows the correlation of serum Calcitonin (CT) and lung IL-33 levels in the chronic HDM model.

[0053] Figures 5A, 5B and 5C: Show the parameters affected by anti-IL-33 treatment in chronic HDM model. Fig. 5A: Frequency of lung neutrophils; Fig. 5B: Frequency of lung eosinophils; Fig. 5C: lung IL-5 levels.

[0054] Figures 6A, 6B and 6C: Show the correlation of serum Calcitonin (CT) and the parameters affected by anti-IL-33 treatment in the chronic HDM model. Fig. 6A: correlation between serum CT levels and frequency of lung neutrophils; Fig. 6B: correlation between serum CT levels and frequency of lung eosinophils; Fig. 6C: correlation between serum CT and lung IL-5 protein levels.

[0055] Figures 7A, 7B and 7C: Show the correlation of lung IL-33 levels and the parameters affected by anti-IL-33 treatment in the chronic HDM model. Fig. 7A: correlation between lung IL-33 levels and frequency of lung neutrophils; Fig. 7B: correlation between lung IL-33 levels and frequency of lung eosinophils; Fig. 7C: correlation between lung IL-33 and lung IL-5 protein levels.

DETAILED DESCRIPTION

[0056] Before the present invention is described, it is to be understood that this invention is not limited to particular methods and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0057] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1%. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (*e.g.*, 99.1, 99.2, 99.3, 99.4, etc.). As used herein, the terms "treat", "treating", or the like, mean to alleviate symptoms, eliminate the causation of symptoms either on a temporary or permanent basis, or to prevent or slow the appearance of symptoms of the named disorder or condition.

[0058] Although any methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, the preferred methods and materials are now described.

Biomarkers Associated with an IL-33-Mediated Disease or Disorder

[0059] The present invention includes methods involving the use, quantification, and analysis of biomarkers associated with an IL-33-mediated disease or disorder.

[0060] An "IL-33 mediated disease or disorder" may be selected from any inflammatory disease or disorder such as, but not limited to, asthma, allergy, allergic rhinitis, allergic airway inflammation, atopic dermatitis (AD), chronic obstructive pulmonary disease (COPD), inflammatory bowel disease (IBD), multiple sclerosis, arthritis, psoriasis, eosinophilic esophagitis, eosinophilic pneumonia, eosinophilic psoriasis, hypereosinophilic syndrome, graft-versus-host disease, uveitis, cardiovascular disease, pain, multiple sclerosis, lupus, vasculitis, chronic idiopathic urticaria and Eosinophilic Granulomatosis with Polyangiitis (Churg-Strauss Syndrome).

[0061] The asthma may be selected from the group consisting of allergic asthma, non-allergic asthma, severe refractory asthma, asthma exacerbations, viral-induced asthma or viral-induced asthma exacerbations, steroid resistant asthma, steroid sensitive asthma, eosinophilic asthma or non-eosinophilic asthma and other related disorders characterized by airway inflammation or airway hyperresponsiveness (AHR).

[0062] The COPD may be a disease or disorder associated in part with, or caused by, cigarette smoke, air pollution, occupational chemicals, allergy or airway hyperresponsiveness.

[0063] The allergy may be associated with foods, pollen, mold, dust mites, animals, or animal dander.

[0064] The IBD may be selected from the group consisting of ulcerative colitis, Crohn's Disease, collagenous colitis, lymphocytic colitis, ischemic colitis, diversion colitis, Behcet's syndrome, infective colitis, indeterminate colitis, and other disorders characterized by inflammation of the mucosal layer of the large intestine or colon.

[0065] The arthritis may be selected from the group consisting of osteoarthritis, rheumatoid arthritis and psoriatic arthritis.

[0066] As used herein, the term "an IL-33-mediated disease or disorder-associated biomarker", or a "biomarker associated with an IL-33-mediated disease or disorder" means any biological response, cell type, parameter, protein, polypeptide, enzyme, enzyme activity, metabolite, nucleic acid, carbohydrate, or other biomolecule which is present or detectable in a patient suffering from an IL-33-mediated disease or disorder at a level or amount that is different from (*e.g.*, greater than or less than) the level or amount of the marker present or detectable in a non-IL-33-mediated disease or disorder patient. Exemplary IL-33-mediated disease or disorder-associated biomarkers include, but are not limited to, *e.g.*, polypeptides encoded by the *CALCA* gene, including calcitonin, procalcitonin, or calcitonin gene-related peptide (CGRP). Other biomarkers associated with an IL-33-mediated disease or disorder include, but are not limited to resistin-like alpha (RETNA); chemokine (C-C motif) ligand 8 (Ccl8); serum amyloid A 3 (Saa3); Gm1975 (BC117090); killer cell lectin-like receptor (Klr1); stefin A1 (Csta); membrane-spanning 4-domain (Ms4a8a); chemokine (C-C motif) ligand 11 (Ccl11); and serine (or cysteine) peptides (Serpina3f).

[0067] Methods for detecting and/or quantifying such biomarkers are known in the art; kits for measuring such biomarkers are available from various commercial sources; and various commercial diagnostic laboratories offer services, which provide measurements of such biomarkers as well.

[0068] In one embodiment, the biomarker is calcitonin and the level of calcitonin is increased in a subject suffering from, or prone to developing an IL-33 mediated disease or disorder. In one embodiment, the biomarker is procalcitonin and the level of procalcitonin is increased in a subject suffering from, or prone to developing an IL-33 mediated disease or disorder. In a related embodiment, the biomarker is calcitonin gene-related peptide (CGRP) and the level of CGRP is increased in a subject suffering from, or prone to developing an IL-33 mediated disease or disorder. The level of calcitonin, procalcitonin, or CGRP may be assessed using any method known to those skilled in the art. Methods of detection include an immunoassay using any label for detection, such as a radioisotopic label, an enzymatic label, or a chemiluminescent label. The interpretation of the results must take into account the method used, the subject's age, gender and weight (See d'Herbomez, M. *et. al.*, (2007), Eur. J. Endocrinology 157:749-755). Generally, a "normal" reference value of calcitonin in males is less than about 16 pg/mL

and less than about 8 pg/mL for females. More recently, Camacho *et.al.* developed a new immunofluorometric assay for serum calcitonin and have validated it in samples from 794 patients. The results obtained using this assay showed that the normal cut-off range for males was less than 11.1 pg/mL and less than 5.5 pg/mL for females. (See Camacho, *et. al.*, (2014), Eur. Thyroid J., Jun;3(2):117-24. A "reference" range of procalcitonin in adults and children older than 72 hours is about 0.15 ng/mL or less. In healthy adults, the reference range of procalcitonin is below the level of detection (See Dandona, P. *et al.* (1994), J. Clin. Endocrinol. Metab. Dec. 79(6):1605-8.). A normal range of CGRP in healthy individuals is generally below about 45 pg/ml. As such, a value above that may be considered indicative of the presence of a disease or disorder.

[0069] An "IL-33 associated disease parameter" may include, but not be limited to, an increase in infiltration of neutrophils, eosinophils or ST2+ CD4 T cells to a tissue (*e.g.* lung), goblet cells metaplasia, an increase in ST2 levels in the tissue or an increase in the level of a cytokine, such as IL-1 β , IL-4, IL-5, IL-6, IL-9, IL-13, IL-33, MCP-1, TNF α in the tissue.

[0070] According to certain aspects of the invention, methods for treating an IL-33-mediated disease or disorder are provided which comprise: (a) selecting a subject who exhibits a level of at least one biomarker prior to or at the time of treatment which signifies the disease state, and (b) administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of an IL-33 antagonist. In one embodiment of this aspect of the invention, the subject is selected on the basis of an elevated level of calcitonin. In one embodiment, the IL-33 antagonist is an isolated monoclonal antibody the specifically binds to IL-33, or a receptor based IL-33 antagonist (an IL-33 trap as described herein).

[0071] According to other aspects of the invention, methods for treating an IL-33-mediated disease or disorder are provided which comprise administering to a subject a pharmaceutical composition comprising a therapeutically effective amount of an IL-33 antagonist, wherein administration of the pharmaceutical composition to the subject results in a decrease in at least one biomarker (*e.g.*, calcitonin (Calca), procalcitonin, calcitonin gene-related peptide (CGRP), resistin-like alpha (RETNA), chemokine (C-C motif) ligand 8 (Ccl8), serum amyloid A 3 (Saa3), Gm1975 (BC117090), killer cell lectin-like receptor (Klrk1), stefin A1 (Csta), membrane-spanning 4-domain (Ms4a8a), chemokine (C-C motif) ligand 11 (Ccl11), and serine (or cysteine) peptides (Serpina3f), etc.) at a time after administration of the pharmaceutical composition, as compared to the level of the biomarker in the subject prior to the administration.

[0072] As will be appreciated by a person of ordinary skill in the art, an increase or decrease in a biomarker associated with an IL-33 mediated disease or disorder can be determined by comparing (i) the level of the biomarker measured in a subject at a defined time point after administration of the pharmaceutical composition comprising an IL-33 antagonist to (ii) the level of the biomarker measured in the patient prior to the administration of the pharmaceutical composition comprising an IL-33 antagonist (*i.e.*, the "baseline measurement"). The defined

time point at which the biomarker is measured can be, *e.g.*, at about 4 hours, 8 hours, 12 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 14 days, 15 days, 20 days, 35 days, 40 days, 50 days, 55 days, 60 days, 65 days, 70 days, 75 days, 80 days, 84 days, 85 days, or more after administration of the of the pharmaceutical composition comprising an IL-33 antagonist.

[0073] According to certain particular embodiments of the present invention, a subject may exhibit a decrease in the level of one or more of calcitonin, procalcitonin, CGRP, resistin-like alpha (RETNA), chemokine (C-C motif) ligand 8 (Ccl8), serum amyloid A 3 (Saa3), Gm1975 (BC117090), killer cell lectin-like receptor (Kirg1), stefin A1 (Csta), membrane-spanning 4-domain (Ms4a8a), chemokine (C-C motif) ligand 11 (Ccl11), and serine (or cysteine) peptides (Serpina3f) following administration of a pharmaceutical composition comprising an IL-33 antagonist (*e.g.*, an anti-IL-33 antibody, or an IL-33 receptor based antagonist (an IL-33 trap). For example, at about day 4, day 8, day 14, day 15, day 22, day 25, day 29, day 36, day 43, day 50, day 57, day 64, day 71, day 84, or day 85, following administration of a first, second, third or fourth dose of a pharmaceutical composition comprising about an anti-hIL-33 antagonist. The dose of an anti-IL-33 antagonist administered to a patient may vary depending upon the age and the size of the patient, target disease, conditions, route of administration, and the like. The preferred dose is typically calculated according to body weight or body surface area. When an IL-33 antagonist is used for treating a condition or disease associated with IL-33 activity in an adult patient, it may be advantageous to intravenously administer the anti-IL-33 antagonist normally at a dose of about 0.01 to about 20 mg/kg body weight, more preferably about 0.02 to about 7, about 0.03 to about 5, or about 0.05 to about 3 mg/kg body weight.

[0074] The subject, according to the present invention, may exhibit a decrease in at least one of the following biomarkers: a polypeptide encoded by the *CALCA* gene, selected from the group consisting of calcitonin, procalcitonin and CGRP, or may exhibit a decrease in at least one of the following biomarkers: resistin-like alpha (RETNA); chemokine (C-C motif) ligand 8 (Ccl8); serum amyloid A 3 (Saa3); Gm1975 (BC117090); killer cell lectin-like receptor (Kirg1); stefin A1 (Csta); membrane-spanning 4-domain (Ms4a8a); chemokine (C-C motif) ligand 11 (Ccl11); and serine (or cysteine) peptides (Serpina3f), of about 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more from baseline (wherein "baseline" is defined as the level of biomarker in the subject just prior to the first administration). Similarly, at about day 4, day 8, day 14, day 15, day 22, day 25, day 29, day 36, day 43, day 50, day 57, day 64, day 71, day 84 or day 85, following administration of a first, second, third or fourth dose of a pharmaceutical composition of an anti-hIL-33 antagonist, the subject, according to the present invention, may exhibit a decrease in at least one of the biomarkers described above of about 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more from baseline (wherein "baseline" is defined as the level of biomarker in the subject just prior to the first administration).

[0075] The present invention also includes methods for determining whether a subject is a suitable subject (*e.g.*, a subject who is likely to respond favorably to therapy with an IL-33 antagonist) for whom administration of a pharmaceutical composition comprising an IL-33 antagonist would be beneficial. For example, if an individual, prior to receiving a pharmaceutical composition comprising an IL-33 antagonist, exhibits a level of a biomarker associated with an IL-33-mediated disease or disorder, which signifies the disease state, the individual is therefore identified as a suitable patient for whom administration of a pharmaceutical composition of the invention (a composition comprising an anti-IL-33 antagonist) would be beneficial. According to certain exemplary embodiments, an individual may be identified as a good candidate for anti-IL-33 therapy if the individual exhibits one or more of the following: (i) a calcitonin level greater than about 5 pg/mL, greater than about 10 pg/mL, greater than about 20 pg/mL, greater than about 30 pg/mL, greater than about 40 pg/mL, greater than about 50 pg/mL, greater than about 60 pg/mL, greater than about 70 pg/mL, greater than about 80 pg/mL, greater than about 90 pg/mL, greater than about 100 pg/mL, or greater than about 200 pg/mL. Additional criteria, such as other clinical indicators of an IL-33-mediated disease or disorder may be used in combination with any of the biomarkers described herein to identify an individual as a suitable candidate for anti-IL-33 therapy as described elsewhere herein (*e.g.*, increase in infiltration of neutrophils, eosinophils or ST2⁺ CD4 T cells to a tissue (*e.g.* lung), goblet cells metaplasia, an increase in ST2 levels in the tissue or an increase in the level of a cytokine, such as IL-1 β , IL-4, IL-5, IL-6, IL-9, IL-13, IL-33, MCP-1, TNF α in the tissue, as well as interferon gamma).

[0076] In other aspects of the invention, biomarker levels and/or changes in biomarker levels with treatment may have predictive value for efficacy of anti-IL33 therapy with particular anti-IL33 agents.

[0077] An additional aspect of the invention provides methods of determining whether a subject is likely to have, or likely to develop an IL-33 mediated disease or disorder, wherein the method comprises obtaining a biological sample from the subject and measuring the level of at least one of the biomarkers described herein; wherein the elevated level of at least one biomarker as compared to the level of the biomarker from a subject not having an IL-33 mediated disease or disorder, or not likely to develop an IL-33 mediated disease or disorder, identifies the subject as a subject who is likely to have, or develop an IL-33 mediated disease or disorder.

[0078] An additional aspect of the invention features methods of treating an IL-33-mediated disease or disorder in a subject comprising administration of an anti-IL33 antagonist, wherein the subject has been diagnosed with an IL-33-mediated disease or disorder, has been treated with the anti-IL33 antagonist, and has been selected for further treatment with the IL-33 antagonist on the basis of exhibiting reduced expression of a biomarker (*e.g.* calcitonin), wherein the reduced expression of the biomarker is determined based on a comparison to the level of expression of the respective biomarker in the subject prior to treatment with the anti-IL-

33 antagonist. A particular aspect of the invention features methods of treating an IL-33-mediated disease or disorder in a subject comprising administration of an anti-IL33 antagonist, wherein the subject has been diagnosed with an IL-33-mediated disease or disorder, has been treated with the anti-IL33 antagonist, and has been selected for further treatment with the IL-33 antagonist on the basis of exhibiting reduced expression of the biomarker, wherein the reduced expression of the biomarker in comparison to pre-treatment expression levels is equal to or greater than about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% reduction in biomarker expression.

Interleukin-33 Antagonists

[0079] As disclosed in detail above, the present invention includes methods, which comprise administering to a subject in need thereof a therapeutic composition comprising an IL-33 antagonist. As used herein, an "IL-33 antagonist" is any agent, which binds to or interacts with IL-33, or with its receptor and inhibits the normal biological signaling function of IL-33 when IL-33 is expressed on a cell *in vitro* or *in vivo*. Non-limiting examples of categories of IL-33 antagonists include small molecule IL-33 antagonists, anti-IL-33 aptamers, peptide-based IL-33 antagonists (*e.g.*, "peptibody" molecules), antibodies or antigen-binding fragments of antibodies that specifically bind human IL-33, or receptor based IL-33 antagonists (*e.g.* an IL-3 trap), such as those described herein.

[0080] In one embodiment, the present invention includes methods that comprise administering to a patient a human antibody, or an antigen-binding fragment thereof, that binds specifically to hIL-33. As used herein, the term "hIL-33" means a human cytokine that specifically binds to its receptor, ST2. The amino acid sequence of human ST2 is shown in SEQ ID NO: 334. The amino acid sequence of human IL-33 is shown in SEQ ID NO: 306. The nucleic acid encoding human IL-33 is shown in SEQ ID NO: 305. The anti-IL-33 antibodies may be selected from any one or more of those having the amino acid sequences described in Table 1.

[0081] In one embodiment, the present invention includes methods that comprise administering to a patient a receptor based IL-33 antagonist, for example, an IL-33 trap, such as those described herein. Five different exemplary IL-33 traps of the invention were constructed using standard molecular biological techniques. Table 2a sets forth a summary description of the different IL-33 traps and their component parts. Table 2b sets forth the amino acid sequences of the IL-33 traps and their component parts.

[0082] The term "antibody," as used herein, is intended to refer to immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains interconnected by disulfide bonds, as well as multimers thereof (*e.g.*, IgM). Each heavy chain comprises a heavy chain variable region (abbreviated herein as HCVR or V_H) and a heavy chain constant region. The heavy chain constant region comprises three domains, C_H1, C_H2 and C_H3.

Each light chain comprises a light chain variable region (abbreviated herein as LCVR or V_L) and a light chain constant region. The light chain constant region comprises one domain (C_L1). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In different embodiments of the invention, the FRs of the anti-IL-33 antibody (or antigen-binding portion thereof) may be identical to the human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

[0083] The term "antibody," as used herein, also includes antigen-binding fragments of full antibody molecules. The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. Antigen-binding fragments of an antibody may be derived, *e.g.*, from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and optionally constant domains. Such DNA is known and/or is readily available from, *e.g.*, commercial sources, DNA libraries (including, *e.g.*, phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

[0084] Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) $F(ab')_2$ fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.*, an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (*e.g.*, monovalent nanobodies, bivalent nanobodies, etc.), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein.

[0085] An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR, which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a V_H domain associated with a V_L domain, the V_H and V_L domains may be situated relative to one another in any suitable arrangement. For

example, the variable region may be dimeric and contain V_H - V_H , V_H - V_L or V_L - V_L dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric V_H or V_L domain.

[0086] In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody of the present invention include: (i) V_H - C_H1 ; (ii) V_H - C_H2 ; (iii) V_H - C_H3 ; (iv) V_H - C_H1 - C_H2 ; (v) V_H - C_H1 - C_H2 - C_H3 ; (vi) V_H - C_H2 - C_H3 ; (vii) V_H - C_L ; (viii) V_L - C_H1 ; (ix) V_L - C_H2 ; (x) V_L - C_H3 ; (xi) V_L - C_H1 - C_H2 ; (xii) V_L - C_H1 - C_H2 - C_H3 ; (xiii) V_L - C_H2 - C_H3 ; and (xiv) V_L - C_L . In any configuration of variable and constant domains, including any of the exemplary configurations listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may consist of at least 2 (*e.g.*, 5, 10, 15, 20, 40, 60 or more) amino acids which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule. Moreover, an antigen-binding fragment of an antibody of the present invention may comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric V_H or V_L domain (*e.g.*, by disulfide bond(s)).

[0087] As with full antibody molecules, antigen-binding fragments may be monospecific or multispecific (*e.g.*, bispecific). A multispecific antigen-binding fragment of an antibody will typically comprise at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope on the same antigen. Any multispecific antibody format, may be adapted for use in the context of an antigen-binding fragment of an antibody of the present invention using routine techniques available in the art.

[0088] The constant region of an antibody is important in the ability of an antibody to fix complement and mediate cell-dependent cytotoxicity. Thus, the isotype of an antibody may be selected on the basis of whether it is desirable for the antibody to mediate cytotoxicity.

[0089] The term "human antibody", as used herein, is intended to include non-naturally occurring human antibodies. The term includes antibodies that are recombinantly produced in a non-human mammal, or in cells of a non-human mammal. The term is not intended to include antibodies isolated from or generated in a human subject.

[0090] The term "recombinant human antibody," as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further below), antibodies isolated from a recombinant, combinatorial human antibody library (described further below), antibodies isolated from an animal (*e.g.*, a mouse) that is transgenic for human immunoglobulin genes (see *e.g.*, Taylor et al. (1992) Nucl. Acids

Res. 20:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

[0091] Human antibodies can exist in two forms that are associated with hinge heterogeneity. In one form, an immunoglobulin molecule comprises a stable four chain construct of approximately 150-160 kDa in which the dimers are held together by an interchain heavy chain disulfide bond. In a second form, the dimers are not linked via inter-chain disulfide bonds and a molecule of about 75-80 kDa is formed composed of a covalently coupled light and heavy chain (half-antibody). These forms have been extremely difficult to separate, even after affinity purification.

[0092] The frequency of appearance of the second form in various intact IgG isotypes is due to, but not limited to, structural differences associated with the hinge region isotype of the antibody. A single amino acid substitution in the hinge region of the human IgG4 hinge can significantly reduce the appearance of the second form (Angal et al. (1993) Molecular Immunology 30:105) to levels typically observed using a human IgG1 hinge. The instant invention encompasses antibodies having one or more mutations in the hinge, C_H2 or C_H3 region which may be desirable, for example, in production, to improve the yield of the desired antibody form.

[0093] An "isolated antibody," as used herein, means an antibody that has been identified and separated and/or recovered from at least one component of its natural environment. For example, an antibody that has been separated or removed from at least one component of an organism, or from a tissue or cell in which the antibody naturally exists or is naturally produced, is an "isolated antibody" for purposes of the present invention. An isolated antibody also includes an antibody *in situ* within a recombinant cell. Isolated antibodies are antibodies that have been subjected to at least one purification or isolation step. According to certain embodiments, an isolated antibody may be substantially free of other cellular material and/or chemicals.

[0094] The term "specifically binds," or the like, means that an antibody or antigen-binding fragment thereof forms a complex with an antigen that is relatively stable under physiologic conditions. Methods for determining whether an antibody specifically binds to an antigen are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like. For example, an antibody that "specifically binds" IL-33, as used in the context of

the present invention, includes antibodies that bind IL-33 or portion thereof with a K_D of less than about 1000 nM, less than about 500 nM, less than about 300 nM, less than about 200 nM, less than about 100 nM, less than about 90 nM, less than about 80 nM, less than about 70 nM, less than about 60 nM, less than about 50 nM, less than about 40 nM, less than about 30 nM, less than about 20 nM, less than about 10 nM, less than about 5 nM, less than about 4 nM, less than about 3 nM, less than about 2 nM, less than about 1 nM or less than about 0.5 nM, as measured in a surface plasmon resonance assay. An isolated antibody that specifically binds human IL-33 may, however, have cross-reactivity to other antigens, such as IL-33 molecules from other (non-human) species.

[0095] The anti-IL-33 antibodies useful for the methods of the present invention may comprise one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains as compared to the corresponding germline sequences from which the antibodies were derived. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases. The present invention includes methods involving the use of antibodies, and antigen-binding fragments thereof, which are derived from any of the amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework and/or CDR regions are mutated to the corresponding residue(s) of the germline sequence from which the antibody was derived, or to the corresponding residue(s) of another human germline sequence, or to a conservative amino acid substitution of the corresponding germline residue(s) (such sequence changes are referred to herein collectively as "germline mutations"). A person of ordinary skill in the art, starting with the heavy and light chain variable region sequences disclosed herein, can easily produce numerous antibodies and antigen-binding fragments which comprise one or more individual germline mutations or combinations thereof. In certain embodiments, all of the framework and/or CDR residues within the V_H and/or V_L domains are mutated back to the residues found in the original germline sequence from which the antibody was derived. In other embodiments, only certain residues are mutated back to the original germline sequence, *e.g.*, only the mutated residues found within the first 8 amino acids of FR1 or within the last 8 amino acids of FR4, or only the mutated residues found within CDR1, CDR2 or CDR3. In other embodiments, one or more of the framework and/or CDR residue(s) are mutated to the corresponding residue(s) of a different germline sequence (*i.e.*, a germline sequence that is different from the germline sequence from which the antibody was originally derived). Furthermore, the antibodies of the present invention may contain any combination of two or more germline mutations within the framework and/or CDR regions, *e.g.*, wherein certain individual residues are mutated to the corresponding residue of a particular germline sequence while certain other residues that differ from the original germline sequence are maintained or are mutated to the corresponding residue of a different germline sequence. Once obtained, antibodies and antigen-binding fragments that contain one

or more germline mutations can be easily tested for one or more desired property such as, improved binding specificity, increased binding affinity, improved or enhanced antagonistic or agonistic biological properties (as the case may be), reduced immunogenicity, etc. The use of antibodies and antigen-binding fragments obtained in this general manner are encompassed within the present invention.

[0096] The present invention also includes methods involving the use of anti-IL-33 antibodies comprising variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more conservative substitutions. For example, the present invention includes the use of anti-IL-33 antibodies having HCVR, LCVR, and/or CDR amino acid sequences with, *e.g.*, 10 or fewer, 8 or fewer, 6 or fewer, 4 or fewer, etc. conservative amino acid substitutions relative to any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein.

[0097] The term "surface plasmon resonance," as used herein, refers to an optical phenomenon that allows for the analysis of real-time interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore™ system (Biacore Life Sciences division of GE Healthcare, Piscataway, NJ).

[0098] The term " K_D ," as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction.

[0099] The term "epitope" refers to an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a polypeptide chain. In certain circumstance, an epitope may include moieties of saccharides, phosphoryl groups, or sulfonyl groups on the antigen.

Preparation of Human Antibodies

[0100] Methods for generating human antibodies in transgenic mice are known in the art. Any such known methods can be used in the context of the present invention to make human antibodies that specifically bind to human IL-33.

[0101] Using VELOCIMMUNE™ technology (see, for example, US 6,596,541, Regeneron Pharmaceuticals) or any other known method for generating monoclonal antibodies, high affinity chimeric antibodies to IL-33 are initially isolated having a human variable region and a mouse constant region. The VELOCIMMUNE® technology involves generation of a transgenic mouse having a genome comprising human heavy and light chain variable regions operably linked to endogenous mouse constant region loci such that the mouse produces an antibody comprising a human variable region and a mouse constant region in response to antigenic stimulation. The

DNA encoding the variable regions of the heavy and light chains of the antibody are isolated and operably linked to DNA encoding the human heavy and light chain constant regions. The DNA is then expressed in a cell capable of expressing the fully human antibody.

[0102] Generally, a VELOCIMMUNE® mouse is challenged with the antigen of interest, and lymphatic cells (such as B-cells) are recovered from the mice that express antibodies. The lymphatic cells may be fused with a myeloma cell line to prepare immortal hybridoma cell lines, and such hybridoma cell lines are screened and selected to identify hybridoma cell lines that produce antibodies specific to the antigen of interest. DNA encoding the variable regions of the heavy chain and light chain may be isolated and linked to desirable isotypic constant regions of the heavy chain and light chain. Such an antibody protein may be produced in a cell, such as a CHO cell. Alternatively, DNA encoding the antigen-specific chimeric antibodies or the variable domains of the light and heavy chains may be isolated directly from antigen-specific lymphocytes.

[0103] Initially, high affinity chimeric antibodies are isolated having a human variable region and a mouse constant region. The antibodies are characterized and selected for desirable characteristics, including affinity, selectivity, epitope, etc, using standard procedures known to those skilled in the art. The mouse constant regions are replaced with a desired human constant region to generate the fully human antibody of the invention, for example wild-type or modified IgG1 or IgG4. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

[0104] In general, the antibodies that can be used in the methods of the present invention possess high affinities, as described above, when measured by binding to antigen either immobilized on solid phase or in solution phase. The mouse constant regions are replaced with desired human constant regions to generate the fully human antibodies of the invention. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

[0105] Specific examples of human antibodies or antigen-binding fragments of antibodies that specifically bind IL-33 which can be used in the context of the methods of the present invention include any antibody or antigen-binding fragment which comprises the three heavy chain CDRs (HCDR1, HCDR2 and HCDR3) contained within a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, and 308. The antibody or antigen-binding fragment may comprise the three light chain CDRs (LCVR1, LCVR2, LCVR3) contained within a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, and 316. Methods and techniques for identifying CDRs within HCVR and LCVR amino acid sequences are well known in the art and can be used to identify CDRs within the specified HCVR and/or LCVR amino acid sequences disclosed herein.

Exemplary conventions that can be used to identify the boundaries of CDRs include, *e.g.*, the Kabat definition, the Chothia definition, and the AbM definition. In general terms, the Kabat definition is based on sequence variability, the Chothia definition is based on the location of the structural loop regions, and the AbM definition is a compromise between the Kabat and Chothia approaches. See, *e.g.*, Kabat, "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1991); Al-Lazikani *et al.*, *J. Mol. Biol.* 273:927-948 (1997); and Martin *et al.*, *Proc. Natl. Acad. Sci. USA* 86:9268-9272 (1989). Public databases are also available for identifying CDR sequences within an antibody.

[0106] In certain embodiments of the present invention, the antibody or antigen-binding fragment thereof comprises the six CDRs (HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3) from the heavy and light chain variable region amino acid sequence pairs (HCVR/LCVR) selected from the group consisting of SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282, 290/298, and 308/316.

[0107] In certain embodiments of the present invention, the antibody or antigen-binding fragment thereof comprises HCVR/LCVR amino acid sequence pairs selected from the group consisting of SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282, 290/298, and 308/316.

[0108] The study summarized in the attached Examples utilized an anti-hIL-33 antibody referred to as H4H9675P. This antibody comprises an HCVR/LCVR amino acid sequence pair having SEQ ID NOs: 274/282, and HCDR1-HCDR2-HCDR3 / LCDR1-LCDR2-LCDR3 domains represented by SEQ ID NOs: 276-278-280 / SEQ ID NOs: 284-286-288. However, the methods of the present invention can be practiced using any anti-IL-33 antibody disclosed herein, as well as variants and antigen-binding fragments of such antibody.

Pharmaceutical Compositions

[0109] The present invention includes methods, which comprise administering an IL-33 antagonist to a patient, wherein the IL-33 antagonist is contained within a pharmaceutical composition. The pharmaceutical compositions of the invention are formulated with suitable carriers, excipients, and other agents that provide suitable transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell *et al.* "Compendium of excipients for parenteral

formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

[0110] The dose of antibody administered to a patient according to the methods of the present invention may vary depending upon the age and the size of the patient, symptoms, conditions, route of administration, and the like. The dose is typically calculated according to body weight or body surface area. Depending on the severity of the condition, the frequency and the duration of the treatment can be adjusted. Effective dosages and schedules for administering pharmaceutical compositions comprising anti-IL-33 antibodies may be determined empirically; for example, patient progress can be monitored by periodic assessment, and the dose adjusted accordingly. Moreover, interspecies scaling of dosages can be performed using well-known methods in the art (*e.g.*, Mordenti *et al.*, 1991, *Pharmaceut. Res.* 8:1351). Specific exemplary doses of anti-IL33 antibodies, and administration regimens involving the same, that can be used in the context of the present invention are disclosed elsewhere herein.

[0111] Various delivery systems are known and can be used to administer the pharmaceutical composition of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, *e.g.*, Wu *et al.*, 1987, *J. Biol. Chem.* 262:4429-4432). Methods of administration include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The composition may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents.

[0112] A pharmaceutical composition of the present invention can be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present invention. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

[0113] Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition of the present invention. Examples include, but are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Bergdorf, Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN),

NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN™, OPTIPEN PRO™, OPTIPEN STARLET™, and OPTICLIK™ (sanofi-aventis, Frankfurt, Germany), to name only a few. Examples of disposable pen delivery devices having applications in subcutaneous delivery of a pharmaceutical composition of the present invention include, but are not limited to the SOLOSTAR™ pen (sanofi-aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly), the SURECLICK™ Autoinjector (Amgen, Thousand Oaks, CA), the PENLET™ (Haselmeier, Stuttgart, Germany), the EPIPEN (Dey, L.P.), and the HUMIRA™ Pen (Abbott Labs, Abbott Park IL), to name only a few.

[0114] In certain situations, the pharmaceutical composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:201). In another embodiment, polymeric materials can be used; see, Medical Applications of Controlled Release, Langer and Wise (eds.), 1974, CRC Pres., Boca Raton, Florida. In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, 1984, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138). Other controlled release systems are discussed in the review by Langer, 1990, Science 249:1527-1533.

[0115] The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by known methods. For example, the injectable preparations may be prepared, *e.g.*, by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (*e.g.*, ethanol), a polyalcohol (*e.g.*, propylene glycol, polyethylene glycol), a nonionic surfactant [*e.g.*, polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there are employed, *e.g.*, sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared can be filled in an appropriate ampoule.

[0116] Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc.

[0117] Exemplary pharmaceutical compositions comprising an anti-IL-33 antibody that can be used in the context of the present invention are disclosed, *e.g.*, in US Patent Application Publication No. 2014/0271658.

Dosage

[0118] The amount of IL-33 antagonist (*e.g.*, anti-IL-33 antibody) administered to a subject according to the methods of the present invention is, generally, a therapeutically effective amount. As used herein, the phrase "therapeutically effective amount" means an amount of IL-33 antagonist that results in a detectable improvement in one or more symptoms or indicia of an IL-33 mediated disease or disorder. A "therapeutically effective amount" also includes an amount of IL-33 antagonist that inhibits, prevents, lessens, or delays the progression of such disease or disorder in a subject.

[0119] In the case of an anti-IL-33 antibody, a therapeutically effective amount can be from about 0.05 mg to about 600 mg, *e.g.*, about 0.05 mg, about 0.1 mg, about 1.0 mg, about 1.5 mg, about 2.0 mg, about 10 mg, about 20 mg, about 30 mg, about 40 mg, about 50 mg, about 60 mg, about 70 mg, about 80 mg, about 90 mg, about 100 mg, about 110 mg, about 120 mg, about 130 mg, about 140 mg, about 150 mg, about 160 mg, about 170 mg, about 180 mg, about 190 mg, about 200 mg, about 210 mg, about 220 mg, about 230 mg, about 240 mg, about 250 mg, about 260 mg, about 270 mg, about 280 mg, about 290 mg, about 300 mg, about 310 mg, about 320 mg, about 330 mg, about 340 mg, about 350 mg, about 360 mg, about 370 mg, about 380 mg, about 390 mg, about 400 mg, about 410 mg, about 420 mg, about 430 mg, about 440 mg, about 450 mg, about 460 mg, about 470 mg, about 480 mg, about 490 mg, about 500 mg, about 510 mg, about 520 mg, about 530 mg, about 540 mg, about 550 mg, about 560 mg, about 570 mg, about 580 mg, about 590 mg, or about 600 mg, of the anti-IL-33 antibody. In certain embodiments, 75 mg, 150 mg, 200 mg, or 300 mg of an anti-IL-33 antibody is administered to a subject.

[0120] The amount of IL-33 antagonist contained within the individual doses may be expressed in terms of milligrams of antibody per kilogram of patient body weight (*i.e.*, mg/kg). For example, the IL-33 antagonist may be administered to a patient at a dose of about 0.0001 to about 10 mg/kg of patient body weight.

Combination Therapies

[0121] The methods of the present invention, according to certain embodiments, comprise administering to the subject one or more additional therapeutic agents in combination with the IL-33 antagonist. As used herein, the expression "in combination with" means that the additional therapeutic agents are administered before, after, or concurrent with the pharmaceutical composition comprising the IL-33 antagonist.

[0122] For example, when administered "before" the pharmaceutical composition comprising the IL-33 antagonist, the additional therapeutic agent may be administered about 72 hours, about 60 hours, about 48 hours, about 36 hours, about 24 hours, about 12 hours, about 10 hours, about 8 hours, about 6 hours, about 4 hours, about 2 hours, about 1 hour, about 30 minutes, about 15 minutes or about 10 minutes prior to the administration of the pharmaceutical

composition comprising the IL-33 antagonist. When administered "after" the pharmaceutical composition comprising the IL-33 antagonist, the additional therapeutic agent may be administered about 10 minutes, about 15 minutes, about 30 minutes, about 1 hour, about 2 hours, about 4 hours, about 6 hours, about 8 hours, about 10 hours, about 12 hours, about 24 hours, about 36 hours, about 48 hours, about 60 hours or about 72 hours after the administration of the pharmaceutical composition comprising the IL-33 antagonist.

[0123] Administration "concurrent" with the pharmaceutical composition comprising the IL-33 antagonist means that the additional therapeutic agent is administered to the subject in a separate dosage form within less than 5 minutes (before, after, or at the same time) of Administration of the pharmaceutical composition comprising the IL-33 antagonist, or administered to the subject as a single combined dosage formulation comprising both the additional therapeutic agent and the IL-33 antagonist.

[0124] The present invention includes methods of treating an IL-33 mediated disease or disorder, which comprise administering to a patient in need of such treatment an anti-hIL-33 antibody, or an IL-33 trap in combination with at least one additional therapeutic agent. Examples of additional therapeutic agents which can be administered in combination with an anti-hIL-33 antibody in the practice of the methods of the present invention include, but are not limited to a non-steroidal anti-inflammatory (NSAID), a steroid, a corticosteroid (inhaled or topical), an immunosuppressant (*e.g.* cyclophosphamide), an anticholinergic agent (*e.g.* tiotropium), a muscarinic agent (*e.g.* glycopyrronium), a phosphodiesterase inhibitor (*e.g.* theophylline, roflumilast, cilomilast), a beta blocker, cyclosporine, tacrolimus, pimecrolimus, azathioprine, methotrexate, cromolyn sodium, a proteinase inhibitor, a bronchial dilator, a beta-2-agonist, an antihistamine, epinephrine, a decongestant, a leukotriene inhibitor, a mast cell inhibitor, a thymic stromal lymphopoietin (TSLP) antagonist, a TNF antagonist, an IgE antagonist, an IL-1 antagonist, an IL-4 or IL-4R antagonist, an IL-13 or IL-13R antagonist, an IL-4/IL-13 dual antagonist, an IL-5 antagonist, an IL-6 or IL-6R antagonist, an antagonist of IL-8, an IL-9 antagonist, an IL-12/23 antagonist, an IL-22 antagonist, an IL-17 antagonist, an IL-31 antagonist, an oral PDE4 inhibitor, an IL-25 antagonist and another IL-33 antagonist or a different antibody or receptor based antagonist to IL-33, and any other compound known to treat, prevent, or ameliorate an IL-33 mediated disease or disorder in a human subject. For example, for concurrent administration, a pharmaceutical formulation can be made which contains both an anti-hIL-33 antibody and at least one additional therapeutic agent. The amount of the additional therapeutic agent that is administered in combination with the anti-hIL-33 antibody in the practice of the methods of the present invention can be easily determined using routine methods known and readily available in the art.

Administration Regimens

[0125] The present invention includes methods comprising administering to a subject a

pharmaceutical composition comprising an IL-33 antagonist at a dosing frequency of about four times a week, twice a week, once a week, once every two weeks, once every three weeks, once every four weeks, once every five weeks, once every six weeks, once every eight weeks, once every twelve weeks, or less frequently so long as a therapeutic response is achieved. In certain embodiments involving the administration of a pharmaceutical composition comprising an anti-IL-33 antibody, once a week dosing at an amount of about 0.01 to about 20 mg/kg body weight, more preferably about 0.02 to about 7, about 0.03 to about 5, or about 0.05 to about 3 mg/kg body weight.

[0126] According to certain embodiments of the present invention, multiple doses of an IL-33 antagonist may be administered to a subject over a defined time course. The methods according to this aspect of the invention comprise sequentially administering to a subject multiple doses of an IL-33 antagonist. As used herein, "sequentially administering" means that each dose of IL-33 antagonist is administered to the subject at a different point in time, *e.g.*, on different days separated by a predetermined interval (*e.g.*, hours, days, weeks or months). The present invention includes methods which comprise sequentially administering to the patient a single initial dose of an IL-33 antagonist, followed by one or more secondary doses of the IL-33 antagonist, and optionally followed by one or more tertiary doses of the IL-33 antagonist.

[0127] The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of the IL-33 antagonist. Thus, the "initial dose" is the dose which is administered at the beginning of the treatment regimen (also referred to as the "baseline dose"); the "secondary doses" are the doses which are administered after the initial dose; and the "tertiary doses" are the doses which are administered after the secondary doses. The initial, secondary, and tertiary doses may all contain the same amount of IL-33 antagonist, but generally may differ from one another in terms of frequency of administration. In certain embodiments, however, the amount of IL-33 antagonist contained in the initial, secondary and/or tertiary doses varies from one another (*e.g.*, adjusted up or down as appropriate) during the course of treatment. In certain embodiments, two or more (*e.g.*, 2, 3, 4, or 5) doses are at the beginning of the treatment regimen as "loading doses" followed by subsequent doses that are administered on a less frequent basis (*e.g.*, "maintenance doses").

[0128] In one exemplary embodiment of the present invention, each secondary and/or tertiary dose is administered 1 to 14 (*e.g.*, 1, 1½, 2, 2½, 3, 3½, 4, 4½, 5, 5½, 6, 6½, 7, 7½, 8, 8½, 9, 9½, 10, 10½, 11, 11½, 12, 12½, 13, 13½, 14, 14½, or more) weeks after the immediately preceding dose. The phrase "the immediately preceding dose," as used herein, means, in a sequence of multiple administrations, the dose of IL-33 antagonist which is administered to a patient prior to the administration of the very next dose in the sequence with no intervening doses.

[0129] The methods according to this aspect of the invention may comprise administering to a patient any number of secondary and/or tertiary doses of an IL-33 antagonist. For example, in

certain embodiments, only a single secondary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) secondary doses are administered to the patient. Likewise, in certain embodiments, only a single tertiary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) tertiary doses are administered to the patient.

[0130] In embodiments involving multiple secondary doses, each secondary dose may be administered at the same frequency as the other secondary doses. For example, each secondary dose may be administered to the patient 1 to 2 weeks after the immediately preceding dose. Similarly, in embodiments involving multiple tertiary doses, each tertiary dose may be administered at the same frequency as the other tertiary doses. For example, each tertiary dose may be administered to the patient 2 to 4 weeks after the immediately preceding dose. Alternatively, the frequency at which the secondary and/or tertiary doses are administered to a patient can vary over the course of the treatment regimen. The frequency of administration may also be adjusted during the course of treatment by a physician depending on the needs of the individual patient following clinical examination.

[0131] The present invention includes administration regimens comprising an up-titration option (also referred to herein as "dose modification"). As used herein, an "up-titration option" means that, after receiving a particular number of doses of an IL-33 inhibitor, if a patient has not achieved a specified improvement in one or more defined therapeutic parameters (*e.g.*, at least a 20% improvement), or otherwise exhibits a clear lack of efficacy in the opinion of a physician or health care provider, the dose of the IL-33 inhibitor is thereafter increased. For example, in the case of a therapeutic regimen comprising administration of 150 mg doses of an anti-IL-33 antibody to a patient at a frequency of once every two weeks, if after 8, 10, 12, 14, 16 or more weeks, the patient has not achieved at least a 20% improvement in one parameter, or if the patient exhibits a clear lack of efficacy in the opinion of a physician or other health care provider, then the dose of anti-IL-33 antibody is increased to *e.g.*, 200 mg, 300 mg, or more, administered once every two weeks thereafter (*e.g.*, starting at week 10, 12, 14, 16, 18, or later).

Treatment Populations

[0132] The present invention includes methods, which comprise administering to a subject in need thereof a therapeutic composition comprising an IL-33 antagonist. As used herein, the expression "a subject in need thereof" means a human or non-human animal that exhibits one or more symptoms or indicia of an IL-33 mediated disease or disorder, such as those described herein (*e.g.*, inflammation, eosinophilia in the lungs, neutrophil infiltration into the lungs, elevated levels of certain cytokines in the lungs, such as, but not limited to IL-5, etc.) and/or who has been diagnosed with any of the IL-33 mediated disease or disorders described herein.

EXAMPLES

[0133] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1. Generation of Human Antibodies to human IL-33

[0134] An immunogen comprising human IL-33 was administered directly, with an adjuvant to stimulate the immune response, to a VELOCIMMUNE® mouse comprising DNA encoding human Immunoglobulin heavy and kappa light chain variable regions. The antibody immune response was monitored by an IL-33-specific immunoassay. When a desired immune response was achieved splenocytes were harvested and fused with mouse myeloma cells to preserve their viability and form hybridoma cell lines. The hybridoma cell lines were screened and selected to identify cell lines that produce IL-33-specific antibodies. Using this technique several anti-IL-33 chimeric antibodies (*i.e.*, antibodies possessing human variable domains and mouse constant domains) were obtained; exemplary antibodies generated in this manner were designated as follows: H1M9559N, H1M9566N, H1M9568N and H1M9565N. The human variable domains from the chimeric antibodies were subsequently cloned onto human constant domains to make fully human anti-IL-33 antibodies as described herein.

[0135] Anti-IL-33 antibodies were also isolated directly from antigen-positive B cells without fusion to myeloma cells, as described in US 2007/0280945A1. Using this method, several fully human anti-IL-33 antibodies (*i.e.*, antibodies possessing human variable domains and human constant domains) were obtained; exemplary antibodies generated in this manner were designated as follows: H4H9629P, H4H9633P, H4H6940P, H4H9659P, H4H9660P, H4H9662P, H4H9663P, H4H9664P, H4H9665P, H4H9666P, H4H9667P, H4H9670P, H4H9671P, H4H9672P, H4H9675P, and H4H9676P.

[0136] Certain biological properties of the exemplary anti-IL-33 antibodies generated in accordance with the methods of this Example are described in detail in the Examples set forth below.

Example 2. Heavy and Light Chain Variable Region Amino Acid Sequences

[0137] Table 1 sets forth the heavy and light chain variable region amino acid sequence pairs, and CDR sequences, of selected anti-IL-33 antibodies and their corresponding antibody identifiers.

Table 1

Antibody Designation	SEQ ID NOs:							
	HCVR	HCDR1	HCDR2	HCDR3	LCVR	LCDR1	LCDR2	LCDR3
H1M9559N	2	4	6	8	10	12	14	16
H1M9566N	18	20	22	24	26	28	30	32
H1M9568N	34	36	38	40	42	44	46	48
H4H9629P	50	52	54	56	58	60	62	64
H4H9633P	66	68	70	72	74	76	78	80
H4H9640P	82	84	86	88	90	92	94	96
H4H9659P	98	100	102	104	106	108	110	112
H4H9660P	114	116	118	120	122	124	126	128
H4H9662P	130	132	134	136	138	140	142	144
H4H9663P	146	148	150	152	154	156	158	160
H4H9664P	162	164	166	168	170	172	174	176
H4H9665P	178	180	182	184	186	188	190	192
H4H9666P	194	196	198	200	202	204	206	208
H4H9667P	210	212	214	216	218	220	222	224
H4H9670P	226	228	230	232	234	236	238	240
H4H9671P	242	244	246	248	250	252	254	256
H4H9672P	258	260	262	264	266	268	270	272
H4H9675P	274	276	278	280	282	284	286	288
H4H9676P	290	292	294	296	298	300	302	304
H1M9565N	308	310	312	314	316	318	320	322

[0138] Antibodies are typically referred to herein according to the following nomenclature: Fc prefix (*e.g.* "H1M," or "H4H"), followed by a numerical identifier (*e.g.* "9559," "9566," or "9629" as shown in Table 1), followed by a "P," or "N" suffix. Thus, according to this nomenclature, an antibody may be referred to herein as, *e.g.*, "H1M9559N," "H1M9566N," "H4H9629P," etc. The H1M and H4H prefixes on the antibody designations used herein indicate the particular Fc region isotype of the antibody. For example, an "H1M" antibody has a mouse IgG1 Fc, whereas an "H4H" antibody has a human IgG4 Fc. As will be appreciated by a person of ordinary skill in the art, an antibody having a particular Fc isotype can be converted to an antibody with a different Fc isotype (*e.g.*, an antibody with a mouse IgG1 Fc can be converted to an antibody with a human IgG4, etc.), but in any event, the variable domains (including the CDRs) – which are indicated by the numerical identifiers shown in Table 1 – will remain the same, and the binding properties are expected to be identical or substantially similar regardless of the nature of the Fc domain.

Example 3. Construction of IL-33 Traps

[0139] Five different exemplary IL-33 antagonists (IL-33 traps) of the invention were constructed using standard molecular biological techniques. The first IL-33 antagonist (hST2-hFc, SEQ ID NO:323) consists of the soluble extracellular region of human ST2 (SEQ ID NO:327) fused at its C-terminus to the N-terminus of a human IgG1 Fc region (SEQ ID

NO:331). The second IL-33 antagonist (hST2-mFc, SEQ ID NO:324) consists of the soluble extracellular region of human ST2 (SEQ ID NO:327) fused at its C-terminus to the N-terminus of a mouse IgG2a Fc region (SEQ ID NO:332). The third IL-33 antagonist (hST2-hIL1RAcP-mFc, SEQ ID NO: 325) consists of an in-line fusion having human ST2 (SEQ ID NO:327) at its N-terminus, followed by the extracellular region of human IL-1RAcP (SEQ ID NO:329), followed by a mouse IgG2a Fc (SEQ ID NO:332) at its C-terminus. The fourth IL-33 antagonist (mST2-mIL1RAcP-mFc, SEQ ID NO: 326) consists of an in-line fusion having mouse ST2 (SEQ ID NO:328) at its N-terminus, followed by the extracellular region of mouse IL-1RAcP (SEQ ID NO:330), followed by a mouse IgG2a Fc (SEQ ID NO:332) at its C-terminus. The fifth IL-33 antagonist (hST2-hIL1RAcP-hFc, SEQ ID NO:335) consists of an in line fusion having human ST2 of SEQ ID NO: 327 at its N-terminus, followed by the extracellular region of human IL-1RAcP (SEQ ID NO: 329) followed by a human IgG1 Fc (SEQ ID NO: 331) at its C terminus. Table 2a sets forth a summary description of the different IL-33 antagonists and their component parts. Table 2b sets forth the amino acid sequences of the IL-33 antagonists and their component parts.

Table 2a: Summary of IL-33 Antagonists (IL-33 traps)

IL-33 Antagonist	Amino Acid Sequence of Full Antagonist Molecule	D1 Component	D2 Component	M Component
hST2-hFc	SEQ ID NO:323	human ST2 extracellular (SEQ ID NO:327)	Absent	human IgG1 Fc (SEQ ID NO:331)
hST2-mFc	SEQ ID NO:324	human ST2 extracellular (SEQ ID NO:327)	Absent	mouse IgG2a Fc (SEQ ID NO:332)
hST2-hIL1RAcP-mFc	SEQ ID NO:325	human ST2 extracellular (SEQ ID NO:327)	human IL-1RAcP extracellular (SEQ ID NO:329)	mouse IgG2a Fc (SEQ ID NO:332)
mST2-mIL1RAcP-mFc	SEQ ID NO:326	mouse ST2 extracellular (SEQ ID NO:328)	mouse IL-1RAcP extracellular (SEQ ID NO:330)	mouse IgG2a Fc (SEQ ID NO:332)
hST2-hIL1RAcP-hFc	SEQ ID NO: 335	human ST2 extracellular (SEQ ID NO:327)	human IL-1RAcP extracellular (SEQ ID NO:329)	human IgG1 Fc (SEQ ID NO:331)

Table 2b: Amino Acid Sequences

Identifier	Sequence
SEQ ID NO:323 (hST2-hFc)	KFSKQSWGLENEALIVRCPRQGKPSYTVDWYYSQTNKSIPTQERNRVFASGQL LKFLPAAVADSGIYTCIVRSPTFNRTGYANVTIYKKQSDCNVPDYLMYSTVSGSE KNSKIYCPTIDLYNWTAPLEWFKNCQALQGSRYRAHKSFLVIDNVMTEADAGDYT CKFIHNENGANYSVTATRSFTVKDEQGFSLFPVIGAPAQNEIKEVEIGKNANLTC SACFGKGTQFLAAVLWQLNGTKITDFGEPRIQQEEGQSQSFSNGLACLDMLVLR ADVKEEDLLLQYDCLALNLHGLRRHTVRLSRKNPIDHHSDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSGGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO:324 (hST2-mFc)	KFSKQSWGLENEALIVRCPRQGKPSYTVDWYYSQTNKSIPTQERNRVFASGQL LKFLPAAVADSGIYTCIVRSPTFNRTGYANVTIYKKQSDCNVPDYLMYSTVSGSE KNSKIYCPTIDLYNWTAPLEWFKNCQALQGSRYRAHKSFLVIDNVMTEADAGDYT CKFIHNENGANYSVTATRSFTVKDEQGFSLFPVIGAPAQNEIKEVEIGKNANLTC SACFGKGTQFLAAVLWQLNGTKITDFGEPRIQQEEGQSQSFSNGLACLDMLVLR ADVKEEDLLLQYDCLALNLHGLRRHTVRLSRKNPIDHHSEPRGPTIKPCPPCKCP APNLLGGPSVFIFPPKIKDVLMSLSPIVTCVVVDVSEDDPDVQISWVFNNEVHT AQTQTHREDYNSTLRVVSALPIQHQQDWMSGKEFKCKVNNKDLPAPIERTISKPK GSVRAPQVYVLPPEEEMTKKQVTLTCMVTDMPEDIYVEWTNNGKTELNYKN TEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPG K
SEQ ID NO:325 (hST2- hIL1RAcP- mFc)	KFSKQSWGLENEALIVRCPRQGKPSYTVDWYYSQTNKSIPTQERNRVFASGQL LKFLPAAVADSGIYTCIVRSPTFNRTGYANVTIYKKQSDCNVPDYLMYSTVSGSE KNSKIYCPTIDLYNWTAPLEWFKNCQALQGSRYRAHKSFLVIDNVMTEADAGDYT CKFIHNENGANYSVTATRSFTVKDEQGFSLFPVIGAPAQNEIKEVEIGKNANLTC SACFGKGTQFLAAVLWQLNGTKITDFGEPRIQQEEGQSQSFSNGLACLDMLVLR ADVKEEDLLLQYDCLALNLHGLRRHTVRLSRKNPIDHHSSERCDDWGLDTMRQI QVFEDEPARIKCPLFEHFLKFNYSTAHSAGLTLIWYWTRQDRDLEEPINFRLPEN RISKEKDVLFWRPTLLNDTGNYTCMLRNTTYCSKVAFPLEVQKDSCFNSPMKL PVHKLYIEYGIQRITCPNVDGYFPSSVKPTITWYMGCYKIQNFNNVIPEGMNL SFL IALISNNGNYTCVVTYPENGRTFHLTRTLTVKVVGSPKNAVPPVIHSPNDHVVE KEPGEELLIPCTVYFSFLMDSRNEVWWTIDGKKPDDITIDVTINESISHSRTEDET RTQILSIKKVTSEDLKRSYVCHARSAKGEVAKAAKVKKVPAPRYTVESGEP PTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMSLSPIVTCVVVDVSEDDPDVQI SWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQQDWMSGKEFKCKVNNKD LPAPIERTISKPKGSVRAPQVYVLPPEEEMTKKQVTLTCMVTDMPEDIYVEWT NNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHN HHTTKSFSRTPGK
SEQ ID NO:326 (mST2- mIL1RAcP- mFc)	SKSSWGLENEALIVRCPRQGRSTYPVEWYYSQTNKSIPTQKRNRI FVSRDRLKF LPARVEDSGIYACVIRSPNLNKTGYLNVTIHKPPSCNIPDYLMYSTVRGSDKNF KITCPTIDLYNWTAPVQWFKNCALQEPRFRAHRSYLFIDNVTHDDEGDYTCQF THAENGNTYIVTATRSFTVEEKGF SMFPVITNPPYNHTMEVEIGKPASIACSACF GKGSHFLADV LWQINKTVVGNFGEARIQEEEGRNESSSNDMDCLTSVLRITGVT EKDLSLEYDCLALNLHGMIRHTIRLRRKQPIDHR SERCDDWGLDTMRQIQVFED EPARIKCPLFEHFLKYNYSTAHSSGLTLIWYWTRQDRDLEEPINFRLPENRISKEK DVLWFRPTLLNDTGNYTCMLRNTTYCSKVAFPLEVQKDSCFN SAMRFPVHKM YIEHGIHKITCPNVDGYFPSSVKPSVTWYKGCTEIVDFHNVLP EGMNLSFFIPLVS NNGNYTCVVTYPENGRLFHLTRTVTVKVVGSPKDALPPQIYSPNDRVVEKEPG EELVIPCKVYFSFIMDSHNEVWWTIDGKKPDDVTVDITINESVSYSSTEDETRTQI LSIKKVTPEDLRRNYVCHARNTKGEAEQA AKVKQKVIPRYTVESGEP CPPCKCPAPNLLGGPSVFIFPPKIKDVLMSLSPIVTCVVVDVSEDDPDVQISWV NNVEVHTAQTQTHREDYNSTLRVVSALPIQHQQDWMSGKEFKCKVNNKDLPAPI ERTISKPKGSVRAPQVYVLPPEEEMTKKQVTLTCMVTDMPEDIYVEWTNNGK

Identifier	Sequence
	TELNYKNTEPVLDSGYSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTK SFSRTPGK
SEQ ID NO:327 (human ST2 extracellular domain)	KFSKQSWGLENEALIVRCPRQGKPSYTVDWYYSQTNKSIPTQERNRVFASGQL LKFLPAAVADSGIYTCIVRSPTFNRTGYANVTIYKKQSDCNVPDYLMYSTVSGSE KNSKIYCPTIDLYNWTAPLEWFKNCQALQGSRYRAHKSFLVIDNVMTEADAGDYT CKFIHNENGANYSVTATRSFTVKDEQGFSLFPVIGAPAQNEIKEVEIGKNANLTC SACFGKGTQFLAAVLWQLNGTKITDFGEPRIQQEEGQNSFSNGLACLDMLVLR ADVKEEDLLLQYDCLALNLHGLRRHTVRLSRKNPIDHHS
SEQ ID NO:328 (mouse ST2 extracellular domain)	SKSSWGLENEALIVRCPRGRSTYPVEWYYSDTNESIPTQKRNRIFVSRDRLKF LPARVEDSGIYACVIRSPNLNKTGYLNVTHKKPPSCNIPDYLMYSTVRGSDKNF KITCPTIDLYNWTAPVQWFKNCKALQEPRFRAHRSYLFIDNVTHDDEGDYTCQF THAENGTYIYVATRSFTVEEKGFMSFPVITNPPYNHTMEVEIGKPASIACSACF GKGSHFLADVWQINKTVVGNFGEARIQEEEGRNESSNDMDCLTSVLRITGVT EKDLSLEYDCLALNLHGMIRHTIRLRRKQPIDHR
SEQ ID NO:329 (human IL1RAcP extracellular domain)	SERCDDWGLDTMRQIQVFEDEPARIKCPLFEHFLKFNYSTAHSAGLTLIWYWTR QDRDLEEPINFRLPENRISKEKDVLWFRPTLLNDTGNYTCMLRNTTYCSKVAFPL EVLQKDSFCFNSPMKLPVHKLYIEYGIQRITCPNVDGYFPSSVKPTITWYMGCYKI QNFNNVIPEGMNLISFLIALISNNGNYTCVVTYPENGRFHLTRTLTVKVVGSPKN AVPPVIHSPNDHVVEYKEPGEELLIPCTVYFSFLMDSRNEVWWTIDGKKPDDITI DVTINESISHSRTEDETRTQILSIKKVTSSEDLKRSYVCHARSAKGEVAKAAKVQK VPAPRYTVE
SEQ ID NO:330 (mouse IL1RAcP extracellular domain)	SERCDDWGLDTMRQIQVFEDEPARIKCPLFEHFLKFNYSTAHSAGLTLIWYWTR QDRDLEEPINFRLPENRISKEKDVLWFRPTLLNDTGNYTCMLRNTTYCSKVAFPL EVLQKDSFCFNSAMRFPVHKMYIEHGIHKITCPNVDGYFPSSVKPSVTWYKGCTE IVDFHNVLPFGMNLISFFIPLVSNNNGNYTCVVTYPENGRFHLTRTLTVKVVGSPK DALPPQIYSPNDRVVEYKEPGEELVIPCKVYFSFIMDSHNEVWWTIDGKKPDDV TVDITINESVSYSSTEDETRRTQILSIKKVTPEDLRRNYVCHARNTKGEAEQAQVK QKVIPPRTVE
SEQ ID NO:331 (human IgG1 Fc)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVVFSCSVMHEALH NHYTQKSLSLSPGK
SEQ ID NO:332 (mouse IgG2a Fc)	EPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMSLSPIVTCVVVDVSEDD PDVQISWVFNNEVHTAQTQTHREDYNSTLRVVSALPIQHQQDWMMSGKEFKCKV NNKDLPAPIERTISKPKGSRAPQVYVLPPEEEMTKKQVTLTCMVTDFMPEDIY VEWTNNGKTELNYKNTEPVLDSGYSYFMYSKLRVEKKNWVERNSYSCSVVHE GLHNHHTTKSFSRTPGK
SEQ ID NO:333 (<i>M. fascicularis</i> IL-33-6His)	SITGISPITESLASLSTYNDQSITFALEDESIEIYVEDLKDKKKDKVLLSYYESQH PSSESGDGVGKMLMVTLSPTKDFWLQANNKEHSVELHKCEKPLPDQAFFVLH NRSFNCSFECKTDPGVFIGVKDNHLALIKVDYSENLGSENILFKLSEILEHHHHH H
SEQ ID NO:335 (hST2- hIL1RAcP-hFc)	KFSKQSWGLENEALIVRCPRQGKPSYTVDWYYSQTNKSIPTQERNRVFASGQL LKFLPAAVADSGIYTCIVRSPTFNRTGYANVTIYKKQSDCNVPDYLMYSTVSGSE KNSKIYCPTIDLYNWTAPLEWFKNCQALQGSRYRAHKSFLVIDNVMTEADAGDYT CKFIHNENGANYSVTATRSFTVKDEQGFSLFPVIGAPAQNEIKEVEIGKNANLTC SACFGKGTQFLAAVLWQLNGTKITDFGEPRIQQEEGQNSFSNGLACLDMLVLR ADVKEEDLLLQYDCLALNLHGLRRHTVRLSRKNPIDHHS SERCDDWGLDTMRQIQVFEDEPARIKCPLFEHFLKFNYSTAHSAGLTLIWYWTR QDRDLEEPINFRLPENRISKEKDVLWFRPTLLNDTGNYTCMLRNTTYCSKVAFPL EVLQKDSFCFNSPMKLPVHKLYIEYGIQRITCPNVDGYFPSSVKPTITWYMGCYKI QNFNNVIPEGMNLISFLIALISNNGNYTCVVTYPENGRFHLTRTLTVKVVGSPKN AVPPVIHSPNDHVVEYKEPGEELLIPCTVYFSFLMDSRNEVWWTIDGKKPDDITI DVTINESISHSRTEDETRTQILSIKKVTSSEDLKRSYVCHARSAKGEVAKAAKVQK VPAPRYTVE

Identifier	Sequence
	RTQILSIKKVTSEDLKRSYVCHARSAKGEVAKAAKVQKVPAPRYTVEDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLS LSPGK

Example 4: Biomarkers for IL-33 Activity

[0140] Studies were done to determine which genes were elevated in mice that overexpressed IL-33. The studies described below summarize the methods and animal models used to determine which genes correlated with IL-33 expression *in vivo* and which genes were modulated following treatment of the animals with an IL-33 antagonist.

Hydrodynamic DNA delivery

[0141] Mouse IL-33 was overexpressed in wild type (WT) mice by hydrodynamic DNA delivery (HDD). For the HDD experiment, WT mice were injected with either 50 µg or 25ug of a plasmid expressing full-length mouse IL-33 (See GenBank accession number NM_001164724; mIL-33) or with 50 µg or 25 ug of the same plasmid devoid of coding sequence (empty vector). Mice were sacrificed 7 days after the HDD injection, and blood, dorsal root ganglia (DRG), heart, kidney, liver, lung and spleen were collected. The top ten genes expressed in these tissues are shown in table 4 below.

Microarray analysis of collected tissue

[0142] Cy3-CTP was incorporated into amplified cRNA from 500 ng of total RNA using QuickAmp RNA Amplification Kit (Agilent). Cy3 labeled cRNA from each sample was then hybridized to a custom Agilent array comprising of 43538 60mer oligos covering mouse transcriptome. The hybridization and wash of the arrays were performed according to the Agilent protocol and arrays were scanned on an Agilent Microarray scanner. The data was extracted from scanned array images using Agilent Feature Extraction Software 9.5.

Serum collection

[0143] Whole blood was collected into Microtainer® tubes by cardiac puncture at the end of the study. Blood was allowed to clot by leaving it undisturbed at room temperature for at least 30 minutes. Clotted blood and cells were pelleted by centrifuging at 18,000 x g for 10 minutes at 4°C in a refrigerated centrifuge. The resulting supernatant, designated serum, was transferred into clean polypropylene plates and processed or stored appropriately.

Determination of IL-33 protein concentrations in the serum or cell lysate extracts

[0144] ELISA kits from R&D systems were used to determine IL-33 concentrations for human

(DY3625) and mouse (DY3626) IL-33 according to the manufacturers instructions.

Determination of Mouse calcitonin (CT) concentrations in the serum or cell lysate extracts

[0145] Mouse calcitonin was measured in the serum of mice using a mouse Calcitonin (CT) ELISA kit from CusaBio (Through ARP) Cat# CSB-E05133m. The assay procedure was carried out according to the manufacturers instructions provided with the kits.

Determination of Mouse IL-5 concentrations in cell lysate extracts

[0146] Cytokine concentrations in the lung protein extracts were measured using a V-Plex custom Mouse multiplex immunoassay kit (MesoScale Discovery, # K152A41), according to the manufacturer's instructions.

House dust mite induced chronic lung inflammation model

[0147] IL-33 HumIn mice were intranasally administered either 50µg house dust mite extract (HDM; Greer, #XPB70D3A2.5) diluted in 20µL of 1X phosphate buffered saline (PBS) or 20µL of 1X PBS for 3 days per week for 15 weeks. A second control group of IL33 HumIn mice were administered 50µg HDM extract diluted in 20µL of 1X PBS for 3 days per week for either 4 or 11 weeks, to assess the severity of the disease at the onset of antibody treatment. Two groups of HDM challenged mice were injected subcutaneously with 25 mg/kg of either an anti-IL-33 antibody, H4H9675P, or an isotype control antibody starting after either 4 or 11 weeks of HDM challenge and then twice per week until the end of the HDM challenge (8 or 4 weeks of antibody treatment). On day 85 of the study, all mice were sacrificed and their lungs were harvested. Experimental dosing and treatment protocol for groups of mice are shown in Table 3.

[0148] IL-33 HumIn mice were intranasally administered either 50µg house dust mite extract (HDM; Greer, #XPB70D3A2.5) diluted in 20µL of 1X phosphate buffered saline (PBS) or 20µL of 1X PBS for 3 days per week for 15 weeks. A second control group of IL33 HumIn mice were administered 50µg HDM extract diluted in 20µL of 1X PBS for 3 days per week for 11 weeks, to assess the severity of the disease at the onset of antibody treatment. Two groups of HDM challenged mice were injected subcutaneously with 25 mg/kg of either an anti-IL-33 antibody, H4H9675P, or an isotype control antibody starting after 11 weeks of HDM challenge and then twice per week until the end of the HDM challenge (8 weeks of antibody treatment). On day 85 of the study, all mice were sacrificed and their lungs were harvested. Experimental dosing and treatment protocol for groups of mice are shown in Table 3.

Table 3: Experimental dosing and treatment protocol for groups of mice

Group	Mice	Intranasal challenge	Length of intranasal challenge	Antibody
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1	IL-33 HumIn mice	1X PBS	15 weeks	None
2	IL-33 HumIn mice	50µg HDM in 20µL 1X PBS	4 or 11 weeks	None
3	IL-33 HumIn mice	50µg HDM in 20µL 1X PBS	15 weeks	None
4	IL-33 HumIn mice	50µg HDM in 20µL 1X PBS	15 weeks	Isotype control antibody
5	IL-33 HumIn mice	50µg HDM in 20µL 1X PBS	15 weeks	Anti-IL-33 antibody (H4H9675P)

Tissue harvest for Gene Expression Analysis

[0149] After exsanguination, lung was removed, placed into tubes containing 400µL of RNA Later (Ambion, Cat# AM720) and stored at -20°C until processing. Tissues were homogenized in TRIzol and chloroform was used for phase separation. The aqueous phase, containing total RNA, was purified using MagMAX™-96 for Microarrays Total RNA Isolation Kit according to manufacturer's specifications. Genomic DNA was removed using MagMAX™Turbo™DNase Buffer and TURBO DNase from the MagMAX kit listed above. mRNA (up to 2.5µg) was reverse-transcribed into cDNA using SuperScript® VILO™ Master Mix. cDNA was diluted to 2ng/µL and 10ng cDNA was amplified with the TaqMan® Gene Expression Master Mix and the relevant probes (mouse B2m, mouse Calca, human IL33) using the ABI 7900HT Sequence Detection System (Applied Biosystems). The B2m probe was used to amplify the beta-2 microglobulin (B2m) gene as an internal control in order to normalize cDNA input differences. Data analysis was performed using Microsoft Excel and GraphPad Prism™ software. Expression of each gene was normalized to B2m expression within the same sample.

Lung harvest for cytokine analysis

[0150] After exsanguination, the cranial and middle lobes of the right lung from each mouse were removed and placed into tubes containing a solution of tissue protein extraction reagent (1X T-PER reagent; Pierce, #78510) supplemented with 1X Halt Protease inhibitor cocktail (Pierce, #78430). All further steps were performed on ice. The volume of T-PER Reagent (containing the protease inhibitor cocktail) was adjusted for each sample to match a 1:8 (w/v) tissue to T-PER ratio. Lung samples were homogenized in the tubes, using the Tissue Lyser II (Qiagen, Cat#85300). The resulting lysates were centrifuged to pellet debris. The supernatants containing the soluble protein extracts were transferred to fresh tubes and stored at 4°C until further analysis.

[0151] Total protein content in the lung protein extracts was measured using a Bradford

assay. For the assay, 10µL of diluted extract samples were plated into 96 well plates in duplicates and mixed with 200µL of 1X Dye Reagent (Biorad, #500-0006). Serial dilutions of bovine serum albumin (BSA; Sigma, #A7979), starting at 700µg/mL in 1X T-Per reagent were used as a standard to determine the protein concentration of the extracts. After a 5-minute incubation at room temperature, absorbance at 595nm was measured on a Molecular Devices SpectraMax M5 plate reader. Data analysis to determine total lung extract protein content based on the BSA standard was performed using GraphPad Prism™ software.

[0152] Each cytokine concentration in lung total protein extracts from all mice in each group was normalized to the total protein content of the extracts measured by the Bradford assay, and expressed for each group as average pg of cytokine per mg of total lung proteins (pg/mg lung protein, ± SD).

Table 4: Top IL-33 Perturbed Genes

DrgIL33	Heart IL33	Kidney IL33	Liver IL33	Lung IL33	Spleen IL33	Gene Symbol	Description
10.2	415.9	33.5	72.7	5.0	86.3	Calca	Calcitonin/calcitonin-gene related peptide (CGRP)
36.2	114.8	51.7	572.8	24.9	13.8	Retnla	Resistin like alpha
6.5	194.0	19.5	152.2	4.5	19.4	Ccl8	Chemokine (C-C motif) ligand 8
6.5	17.8	251.0	14.9	8.0	45.9	Saa3	Serum amyloid A 3
38.3	28.2	8.3	66.6	53.2	29.3	BC117090	Gm1975
4.9	32.8	61.2	40.4	15.3	3.6	Klrg1	Killer cell lectin-like receptor
15.4	11.7	6.8	86.7	27.1	13.8	Csta	Stefin A1
5.7	46.1	18.2	38.4	1.8	4.5	Ms4a8a	Membrane-spanning 4-domain
1.7	19.0	23.3	81.6	9.4	6.6	Ccl11	Chemokine (C-C motif) ligand 11
3.7	17.9	49.0	18.0	25.8	10.5	Serpina3f	Serine (or cysteine) peptides

Lung harvest for pulmonary cell infiltrate analysis

[0153] After exsanguination, the caudal lobe of the right lung from each mouse was removed, placed into a tube containing a solution of 20µg/mL DNase and 0.7U/mL Liberase TH diluted in Hank's Balanced Salt Solution (HBSS) and chopped into pieces that were approximately 2 to 3mm in size. The tubes with the chopped lungs were then incubated in a 37°C water bath for 20 minutes. The reaction was stopped by adding ethylenediaminetetraacetic acid (EDTA) at a final concentration of 10mM. The samples were then transferred to gentleMACS C Tubes. The volume in the C Tubes was brought up to 3mL with MACS buffer and the samples were subsequently dissociated to form single cell suspensions using a gentleMACS dissociator® (Miltenyi Biotec). The tubes were then centrifuged and the resulting pellet was resuspended in 4mL of 1X Red Blood Cell Lysing Buffer to lyse red blood cells. After incubation for 3 minutes at

room temperature, 10mL of 1X DPBS was added to deactivate the red blood cell lysing buffer. The cell suspensions were then centrifuged, and the resulting cell pellets were resuspended in 1mL of 1X DPBS. The resuspended samples were centrifuge-filtered (1 minute at 400 x g) through a 100µm filter plate and approximately 1.5×10^6 cells per well were plated in a 96-well U-bottom plate. Cells were then centrifuged and the cell pellets resuspended in 100µL of Near-IR LIVE/DEAD® Fixable Dead Cell Stain (Invitrogen Cat#: L34976, Lot#: 1647137) diluted at 1:500 in 1X DPBS to determine cell viability. Cells were incubated with the viability dye for 20 minutes at room temperature while protected from light. After one wash in 1X DPBS, cells were incubated in 50µL of MACS buffer containing 10µg/mL of purified rat anti-mouse CD16/CD32 Fc Block, for 10 minutes at 4°C. The cells were then incubated in the appropriate 2x antibody mixture diluted in Brilliant Stain Buffer (described in Table 5) for 30 minutes at 4°C while protected from light. After antibody incubation, the cells were washed twice in MACS buffer, resuspended in BD CytoFix that had been diluted 1:4 in 1X DPBS and then incubated for 15 minutes at 4°C while protected from light. The cells were subsequently washed and resuspended in MACS buffer. Cell suspensions were then filtered into a new U-Bottom plate through an AcroPrep Advance 96 Filter Plate 30-40µm. Sample data were acquired on a LSR Fortessa X-20 cell analyzer using the HTS attachment (BD Biosciences). Data analysis was performed using FlowJo X Software (Tree Star, OR) and statistical analysis was performed using GraphPad Prism™ (GraphPad Software, CA). Eosinophils were defined as live (cell viability dye negative), singlets, CD45⁺, F4/80⁺, Ly6G⁻, CD11c^{lo-int}, SiglecF^{hi}. Neutrophils were defined as live, singlets, CD45⁺, F4/80⁻, Ly6G⁺. Data for eosinophils, and neutrophils were expressed as frequency of live cells.

Table 5: Antibodies Used for Flow Cytometry Analysis

Antibody	Fluorochrome	Manufacturer	Catalog #, Lot #	Final dilution
CD45	Alexa Fluor 700	BioLegend	103128, B191240	1/200
Siglec-F	BV 421	BD	562681, 4234913	1/200
F4/80	PE	BD	56410, 5054900	1/500
Ly6G	BUV395	BD	563978, 4178621	1/200
CD11c	APC	BD	550261, 5016523	1/200

Statistical Analysis

[0154] Statistical analyses were performed using GraphPad Prism™ version 6.0 (GraphPad Software, CA).

[0155] Normality of the data was evaluated using the Kolmogorov-Smirnov test. If data passed the normality test, and standard deviations of the different groups were not statistically different from each other as assessed by the Brown-Forsythe test, results were interpreted by one-way analysis of variance (ANOVA) followed by the Tukey *post hoc* test for multiple

comparisons. If data failed to pass the normality test, or standard deviations were significantly different, results were interpreted using the Kruskal-Wallis test followed by the Dunn's *post hoc* test for multiple comparisons. Differences were considered to be statistically significant when P value < 0.05.

[0156] Correlation coefficient and significance were computed using two-tailed Spearman non-parametric correlation. Correlations were considered to be statistically significant when P value < 0.05.

Summary of Results:

[0157] The results demonstrated that the top ten genes that were perturbed in animals that overexpressed IL-33 were calcitonin (Calca), resistin-like alpha (RETNA), chemokine (C-C motif) ligand 8 (Ccl8), serum amyloid A 3 (Saa3), Gm1975 (BC117090), killer cell lectin-like receptor (Kirg1), stefin A1 (Csta), membrane-spanning 4-domain (Ms4a8a), chemokine (C-C motif) ligand 11 (Ccl11), and serine (or cysteine) peptides (Serpina3f) (See Table 4). Moreover, the data also showed that both serum IL-33 and serum calcitonin were significantly elevated in this mouse model (See Figure 1A for serum IL-33 levels and Figure 1B for serum calcitonin levels). The increase in serum IL-33 correlated with the increase in serum levels of calcitonin.

[0158] In addition, the chronic house dust mite (HDM) challenge model, which was used as a model of severe, progressive lung inflammation following exposure to an allergen, showed that calcitonin gene expression is upregulated in the lungs of mice upon chronic HDM challenge, but the expression of calcitonin is reduced significantly in the lungs of mice that have been treated with an IL-33 antagonist (H4H9675P anti-IL-33 antibody). Treatment with an isotype matched negative control antibody had no effect on the level of calcitonin gene expression (Figure 2).

[0159] In addition, serum calcitonin was also elevated in this HDM mouse model and the level of serum calcitonin correlated with the level of calcitonin seen in the lungs of these mice (See Figure 3). Moreover, the level of serum calcitonin correlated with the elevated levels of IL-33 observed in this HDM mouse model (See Figure 4), suggesting that calcitonin could be used as a potential biomarker for IL-33 in an IL-33 mediated disease process (*e.g.* an allergic response).

[0160] In terms of IL-33 mediated disease parameters, the frequency of lung neutrophils, lung eosinophils and IL5 levels were studied in this HDM model. By 15 weeks after challenge with house dust mite allergen, all three parameters were found to be elevated (See figures 5A (neutrophils), 5B (eosinophils) and 5C (IL-5)). However, mice that received the IL-33 antibody H4H9675P showed significantly fewer neutrophils, eosinophils and IL-5 in their lungs compared to the mice challenged with allergen and left untreated, or administered a negative isotype control antibody (See Figures 5A, 5B and 5C).

[0161] Furthermore, there was a significant correlation between serum calcitonin levels and the three disease parameters studied. Figure 6A shows the correlation between serum calcitonin and lung neutrophils. Figure 6B shows the correlation between serum calcitonin and

lung eosinophils and Figure 6C shows the correlation between serum calcitonin and lung IL-5 levels.

[0162] In addition, there was a significant correlation between lung IL-33 levels and the frequency of lung neutrophils (Figure 7A), lung eosinophils (Figure 7B) and lung IL-5 levels (Figure 7C).

[0163] In summary, the data obtained from the house dust mite model indicate that there is a significant correlation between the level of IL-33, the frequency of at least three disease parameters associated with this model of chronic allergy and the expression of calcitonin. As such, this information suggests that calcitonin may be one biomarker of disease severity and/or progression in a mammal and furthermore, the data suggest that calcitonin may be used to assess the effectiveness of therapy with an IL-33 antagonist as described herein.

[0164] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

What is claimed is:

1. Use of a pharmaceutical composition comprising a therapeutically effective amount of an interleukin-33 antagonist for treating an interleukin-33 (IL-33) mediated disease or disorder in a subject who exhibits an elevated level of at least one biomarker associated with an IL-33 mediated disease or disorder.

2. The use of claim 1, wherein the biomarker is a polypeptide encoded by the *CALCA* gene and is selected from the group consisting of calcitonin, procalcitonin and calcitonin gene-related peptide (CGRP).

3. The use of claim 2, wherein the biomarker is calcitonin.

4. The use of claim 1, wherein the IL-33 antagonist comprises an anti-IL-33 antibody, or an anti-IL-33 receptor based antagonist (an IL-33 trap).

5. The use of claim 4, wherein the anti-IL-33 antibody is an isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds human interleukin-33 (IL-33), wherein the antibody or antigen-binding fragment comprises: (a) the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, and 308; and (b) the CDRs of a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, and 316.

6. The use of either claim 4 or 5, wherein the anti-IL-33 antibody or antigen-binding fragment thereof comprises the heavy and light chain CDRs of a HCVR/LCVR amino acid sequence pair selected from the group consisting of: SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282, 290/298, and 308/316.

7. The use of any of claims 4-6, wherein the antibody or antigen-binding fragment comprises HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively, selected from the group consisting of: SEQ ID NOs: 4-6-8-12-14-16; 20-22-24-28-30-32; 36-38-40-44-46-48; 52-54-56-60-62-64; 68-70-72-76-78-80; 84-86-88-92-94-96; 100-102-104-108-110-112; 116-118-120-124-126-128; 132-134-136-140-142-144; 148-150-152-156-158-160; 164-166-168-172-174-176; 180-182-184-188-190-192; 196-198-200-204-206-208; 212-214-216-220-222-224; 228-230-232-236-238-240; 244-246-248-252-254-256; 260-262-264-268-270-272; 276-278-280-284-286-288; 292-294-296-300-302-304; and 310-312-314-318-320-322.

8. The use of any of claims 4-7, wherein the antibody or antigen-binding fragment comprises: (a) a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, and 308; and (b) a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, and 316.

9. The use of claim 4, wherein the IL-33 receptor based antagonist comprises a first IL-33 binding domain (D1) attached to a multimerizing domain (M), wherein D1 comprises an IL-33-binding portion of an ST2 protein.

10. The use of claim 9, wherein the IL-33 receptor based antagonist further comprises one or more additional IL-33 binding domains selected from the group consisting of D2, D3 and D4.

11. The use of claim 4, wherein the IL-33 receptor based antagonist comprises an IL-33-binding portion of an ST2 protein, an extracellular domain of an IL-1RAcP protein, or other IL-33 binding domain.

12. The use of claim 4, wherein the IL-33 receptor based antagonist is selected from the group consisting of SEQ ID NOs: 323, 324, 325, 326 and 335.

13. The use of any of claims 1-12, wherein the pharmaceutical composition is for use in combination with an effective amount of a second therapeutic agent for reducing at least one symptom of an IL-33 mediated disease or disorder.

14. The use of claim 13, wherein the second therapeutic agent is selected from the group consisting of a non-steroidal anti-inflammatory (NSAID), a corticosteroid, a bronchial dilator, an antihistamine, epinephrine, a decongestant, a thymic stromal lymphopoietin (TSLP) antagonist, an IL-13 antagonist, an IL-4 antagonist, an IL-4/IL-13 dual antagonist, an IL-5 antagonist, an IL-6 antagonist, an IL-12/23 antagonist, an IL-22 antagonist, an IL-25 antagonist, an IL-17 antagonist, an IL-31 antagonist, an oral PDE4 inhibitor and another IL-33 antagonist or a different antibody or receptor based antagonist to IL-33.

15. The use of claim 1, wherein the biomarker associated with an IL-33 mediated disease or disorder in a subject is selected from the group consisting of calcitonin, procalcitonin, calcitonin gene-related peptide (CGRP), resistin-like alpha (RETNA), chemokine (C-C motif) ligand 8 (Ccl8), serum amyloid A 3 (Saa3), Gm1975 (BC117090), killer cell lectin-like receptor (Kirg1), stefin A1 (Csta), membrane-spanning 4-domain (Ms4a8a), chemokine (C-C motif)

ligand 11 (Ccl11), and serine (or cysteine) peptides (Serpina3f).

16. The use of claim 1, wherein the biomarker is calcitonin, and wherein the calcitonin level is increased in the serum of the subject having an IL-33 mediated disease or disorder, and wherein the increase in serum calcitonin correlates with an increased level of calcitonin and IL-33 in the lungs of the subject.

17. The use of claim 16, wherein treating the IL-33 mediated disease or disorder comprises reducing the level of calcitonin in the serum of the subject to a normal level.

18. The use of claim 1, wherein the elevated level of the at least one biomarker corresponds to a mean serum calcitonin level of greater than about 5 pg/mL for females and greater than about 10 pg/mL for males.

19. The use of claim 1, wherein the IL-33 mediated disease or disorder is an inflammatory disease or disorder selected from the group consisting of asthma, allergy, allergic rhinitis, allergic airway inflammation, atopic dermatitis (AD), chronic obstructive pulmonary disease (COPD), inflammatory bowel disease (IBD), multiple sclerosis, arthritis, psoriasis, eosinophilic esophagitis, eosinophilic pneumonia, eosinophilic psoriasis, hypereosinophilic syndrome, graft-versus-host disease, uveitis, cardiovascular disease, pain, multiple sclerosis, lupus, vasculitis, chronic idiopathic urticaria and Eosinophilic Granulomatosis with Polyangiitis (Churg-Strauss Syndrome).

20. A method for identifying a subject suffering from an IL-33 mediated disease or disorder who is likely to respond favorably to therapy with an IL-33 antagonist, the method comprising obtaining a sample from the subject and measuring the level of calcitonin, procalcitonin, or calcitonin gene-related peptide (CGRP) in the sample; wherein an elevated level of calcitonin, procalcitonin, or CGRP as compared to the lower level of calcitonin, procalcitonin, or CGRP in subjects not suffering from an IL-33 mediated disease or disorder, identifies the patient as a patient who is likely to respond favorably to therapy with an IL-33 antagonist.

21. The method of claim 20, wherein the IL-33 mediated disease or disorder is an inflammatory disease or disorder selected from the group consisting of asthma, allergy, allergic rhinitis, allergic airway inflammation, atopic dermatitis (AD), chronic obstructive pulmonary disease (COPD), inflammatory bowel disease (IBD), multiple sclerosis, arthritis, psoriasis, eosinophilic esophagitis, eosinophilic pneumonia, eosinophilic psoriasis, hypereosinophilic syndrome, graft-versus-host disease, uveitis, cardiovascular disease, pain, multiple sclerosis,

lupus, vasculitis, chronic idiopathic urticaria and Eosinophilic Granulomatosis with Polyangiitis (Churg-Strauss Syndrome).

22. The method of claim 20, wherein the IL-33 antagonist is an anti-IL-33 antibody, or an anti-IL-33 receptor based antagonist (an IL-33 trap).

23. The method of claim 22, wherein the anti-IL-33 antibody is an isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds human interleukin-33 (IL-33), wherein the antibody or antigen-binding fragment comprises: (a) the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, and 308; and (b) the CDRs of a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, and 316.

24. The method of either claim 22 or 23, wherein the anti-IL-33 antibody or antigen-binding fragment thereof comprises the heavy and light chain CDRs of a HCVR/LCVR amino acid sequence pair selected from the group consisting of: SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282, 290/298, and 308/316.

25. The method of any of claims 22-24, wherein the antibody or antigen-binding fragment comprises HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively, selected from the group consisting of: SEQ ID NOs: 4-6-8-12-14-16; 20-22-24-28-30-32; 36-38-40-44-46-48; 52-54-56-60-62-64; 68-70-72-76-78-80; 84-86-88-92-94-96; 100-102-104-108-110-112; 116-118-120-124-126-128; 132-134-136-140-142-144; 148-150-152-156-158-160; 164-166-168-172-174-176; 180-182-184-188-190-192; 196-198-200-204-206-208; 212-214-216-220-222-224; 228-230-232-236-238-240; 244-246-248-252-254-256; 260-262-264-268-270-272; 276-278-280-284-286-288; 292-294-296-300-302-304; and 310-312-314-318-320-322.

26. The method of any of claims 22-25, wherein the antibody or antigen-binding fragment comprises: (a) a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, and 308; and (b) a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, and 316.

27. The method of claim 22, wherein the IL-33 receptor based antagonist comprises a

first IL-33 binding domain (D1) attached to a multimerizing domain (M), wherein D1 comprises an IL-33-binding portion of an ST2 protein.

28. The method of claim 27, wherein the IL-33 receptor based antagonist further comprises one or more additional IL-33 binding domains selected from the group consisting of D2, D3 and D4.

29. The method of claim 22, wherein the IL-33 receptor based antagonist comprises an IL-33-binding portion of an ST2 protein, an extracellular domain of an IL-1 RAcP protein, or other IL-33 binding domain.

30. The method of claim 22, wherein the IL-33 receptor based antagonist is selected from the group consisting of SEQ ID NOs: 323, 324, 325, 326 and 335.

31. A method of determining whether a subject is likely to have, or likely to develop an IL-33 mediated disease or disorder, the method comprising obtaining a sample from the subject and measuring in the sample the level of calcitonin, procalcitonin, or CGRP; wherein an elevated level of calcitonin, procalcitonin, or CGRP, as compared to the lower level of calcitonin, procalcitonin, or CGRP in subjects not likely to have, or not likely to develop an IL-33 mediated disease or disorder, identifies the patient as a patient who is likely to have, or develop an IL-33 mediated disease or disorder.

32. The method of claim 31, wherein the IL-33 mediated disease or disorder is an inflammatory disease or disorder selected from the group consisting of asthma, allergy, allergic rhinitis, allergic airway inflammation, atopic dermatitis (AD), chronic obstructive pulmonary disease (COPD), inflammatory bowel disease (IBD), multiple sclerosis, arthritis, psoriasis, eosinophilic esophagitis, eosinophilic pneumonia, eosinophilic psoriasis, hypereosinophilic syndrome, graft-versus-host disease, uveitis, cardiovascular disease, pain, multiple sclerosis, lupus, vasculitis, chronic idiopathic urticaria and Eosinophilic Granulomatosis with Polyangiitis (Churg-Strauss Syndrome).

33. The method of claim 31, wherein the sample from the subject is a solid tissue sample, a cell sample, or a blood sample.

34. The method of claim 33, wherein the solid tissue sample is selected from the group consisting of heart, kidney, liver, lung and spleen.

35. The method of claim 33, wherein the cell sample is a dorsal root ganglion cell

sample, sputum cell sample, bronchoalveolar lavage cell sample, nasal polyps cell sample, fecal cell sample, lung, colon heart, kidney, skin biopsy, or spleen biopsy cell sample.

36. The method of claim 33, wherein the blood sample is whole blood, plasma, or serum.

37. Use of a pharmaceutical composition comprising a therapeutically effective amount of an IL-33 antagonist for treating an IL-33 mediated disease or disorder in a subject that has been diagnosed with an IL-33 mediated disease or disorder and has been selected for treatment with the IL-33 antagonist on the basis of exhibiting enhanced expression of at least one biomarker pre-treatment, wherein the enhanced expression of the biomarker is determined based on a comparison to a reference level of expression of the respective biomarker.

38. The use of claim 37, wherein the IL-33 mediated disease or disorder is an inflammatory disease or disorder selected from the group consisting of asthma, allergy, allergic rhinitis, allergic airway inflammation, atopic dermatitis (AD), chronic obstructive pulmonary disease (COPD), inflammatory bowel disease (IBD), multiple sclerosis, arthritis, psoriasis, eosinophilic esophagitis, eosinophilic pneumonia, eosinophilic psoriasis, hypereosinophilic syndrome, graft-versus-host disease, uveitis, cardiovascular disease, pain, multiple sclerosis, lupus, vasculitis, chronic idiopathic urticaria and Eosinophilic Granulomatosis with Polyangiitis (Churg-Strauss Syndrome).

39. The use of claim 37, wherein the biomarker associated with an IL-33 mediated disease or disorder in a subject is selected from the group consisting of calcitonin, procalcitonin, CGRP, resistin-like alpha (RETNA), chemokine (C-C motif) ligand 8 (Ccl8), serum amyloid A 3 (Saa3), Gm1975 (BC117090), killer cell lectin-like receptor (Klrk1), stefin A1 (Csta), membrane-spanning 4-domain (Ms4a8a), chemokine (C-C motif) ligand 11 (Ccl11), and serine (or cysteine) peptides (Serpina3f).

40. The use of claim 37, wherein the IL-33 antagonist comprises an anti-IL-33 antibody, or an anti-IL-33 receptor based antagonist (an IL-33 trap).

41. The use of claim 40, wherein the anti-IL-33 antibody is an isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds human interleukin-33 (IL-33), wherein the antibody or antigen-binding fragment comprises: (a) the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, and 308; and (b) the CDRs of a light chain variable region (LCVR) having an amino acid sequence selected from the group

consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, and 316.

42. The use of either claim 40 or 41, wherein the anti-IL-33 antibody or antigen-binding fragment thereof comprises the heavy and light chain CDRs of a HCVR/LCVR amino acid sequence pair selected from the group consisting of: SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282, 290/298, and 308/316.

43. The use of any of claims 40-42, wherein the antibody or antigen-binding fragment comprises HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively, selected from the group consisting of: SEQ ID NOs: 4-6-8-12-14-16; 20-22-24-28-30-32; 36-38-40-44-46-48; 52-54-56-60-62-64; 68-70-72-76-78-80; 84-86-88-92-94-96; 100-102-104-108-110-112; 116-118-120-124-126-128; 132-134-136-140-142-144; 148-150-152-156-158-160; 164-166-168-172-174-176; 180-182-184-188-190-192; 196-198-200-204-206-208; 212-214-216-220-222-224; 228-230-232-236-238-240; 244-246-248-252-254-256; 260-262-264-268-270-272; 276-278-280-284-286-288; 292-294-296-300-302-304; and 310-312-314-318-320-322.

44. The use of any of claims 40-43, wherein the antibody or antigen-binding fragment comprises: (a) a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, and 308; and (b) a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, and 316.

45. The use of claim 40, wherein the IL-33 receptor based antagonist comprises a first IL-33 binding domain (D1) attached to a multimerizing domain (M), wherein D1 comprises an IL-33-binding portion of an ST2 protein.

46. The use of claim 45, wherein the IL-33 receptor based antagonist further comprises one or more additional IL-33 binding domains selected from the group consisting of D2, D3 and D4.

47. The use of claim 40, wherein the IL-33 receptor based antagonist comprises an IL-33-binding portion of an ST2 protein, an extracellular domain of an IL-1RAcP protein, or other IL-33 binding domain.

48. The use of claim 40, wherein the IL-33 receptor based antagonist is selected from the group consisting of SEQ ID NOs: 323, 324, 325, 326 and 335.

49. The use of claim 40, wherein the pharmaceutical composition is for use in combination with a second therapeutic agent for reducing at least one symptom of an IL-33 mediated disease or disorder.

50. The use of claim 49, wherein the second therapeutic agent is selected from the group consisting of a non-steroidal anti-inflammatory (NSAID), a steroid, a corticosteroid (inhaled or topical), an immunosuppressant (*e.g.* cyclophosphamide), an anticholinergic agent (*e.g.* tiotropium), a muscarinic agent (*e.g.* glycopyrronium), a phosphodiesterase inhibitor (*e.g.* theophylline, roflumilast, cilomilast), a beta blocker, cyclosporine, tacrolimus, pimecrolimus, azathioprine, methotrexate, cromolyn sodium, a proteinase inhibitor, a bronchial dilator, a beta-2-agonist, an antihistamine, epinephrine, a decongestant, a leukotriene inhibitor, a mast cell inhibitor, a thymic stromal lymphopoietin (TSLP) antagonist, a TNF antagonist, an IgE antagonist, an IL-1 antagonist, an IL-4 or IL-4R antagonist, an IL-13 or IL-13R antagonist, an IL-4/IL-13 dual antagonist, an IL-5 antagonist, an IL-6 or IL-6R antagonist, an antagonist of IL-8, an IL-9 antagonist, an IL-12/23 antagonist, an IL-22 antagonist, an IL-17 antagonist, an IL-31 antagonist, an IL-33 antagonist, an oral PDE4 inhibitor, and a different antibody to IL-25.

51. A serum biomarker for measuring the presence of an IL-33 mediated disease or disorder in a subject, wherein a level of the serum biomarker correlates with enhanced IL-33 levels *in vivo*, and wherein the serum biomarker is a polypeptide encoded by the *CALCA* gene and is selected from the group consisting of calcitonin, procalcitonin, and CGRP.

52. The serum biomarker of claim 51, for measuring the presence of an IL-33 mediated disease or disorder in a subject, wherein the level of the serum biomarker correlates with enhanced IL-33 levels *in vivo*, and wherein the biomarker is calcitonin.

Serum mIL-33

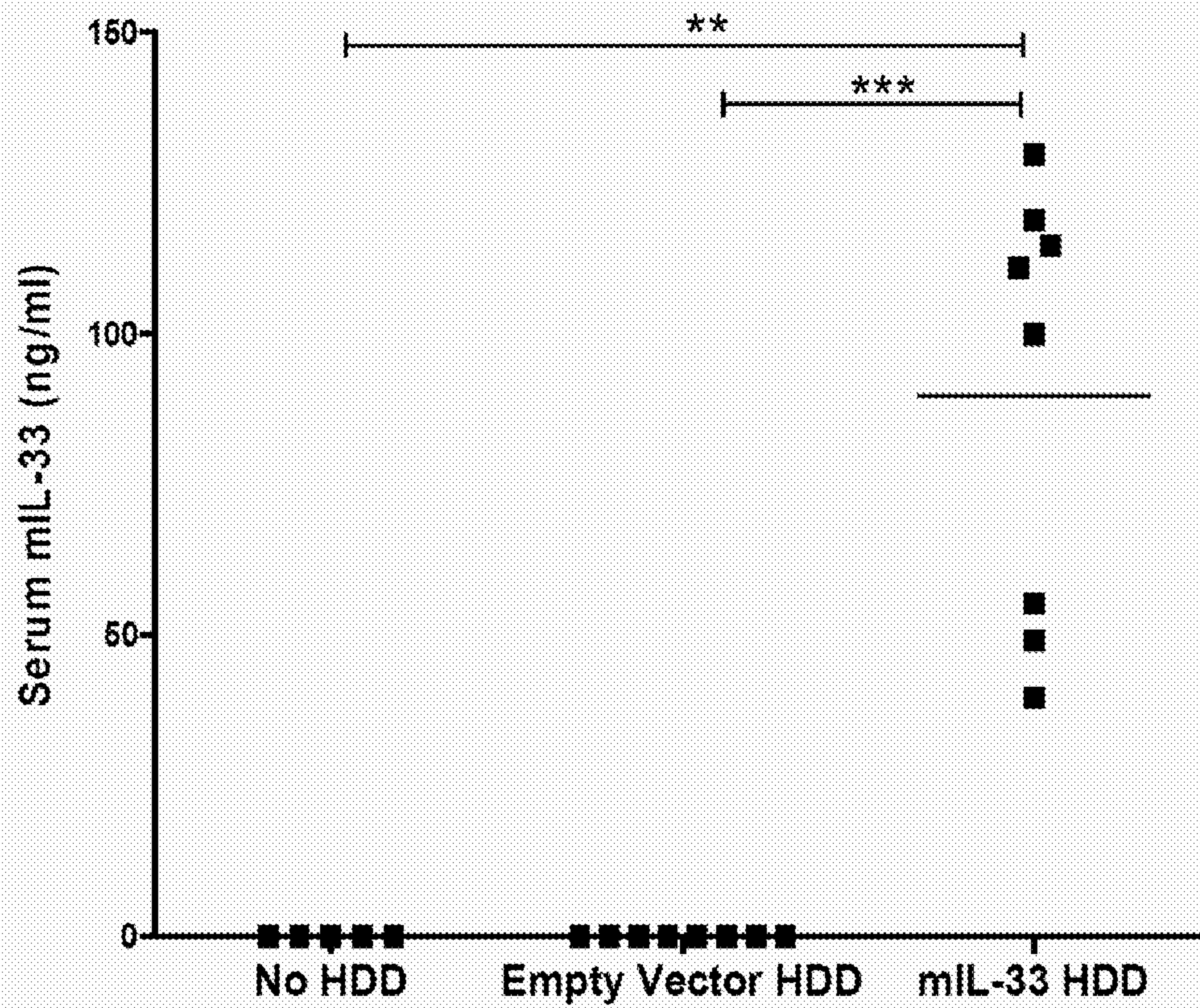


Figure 1A

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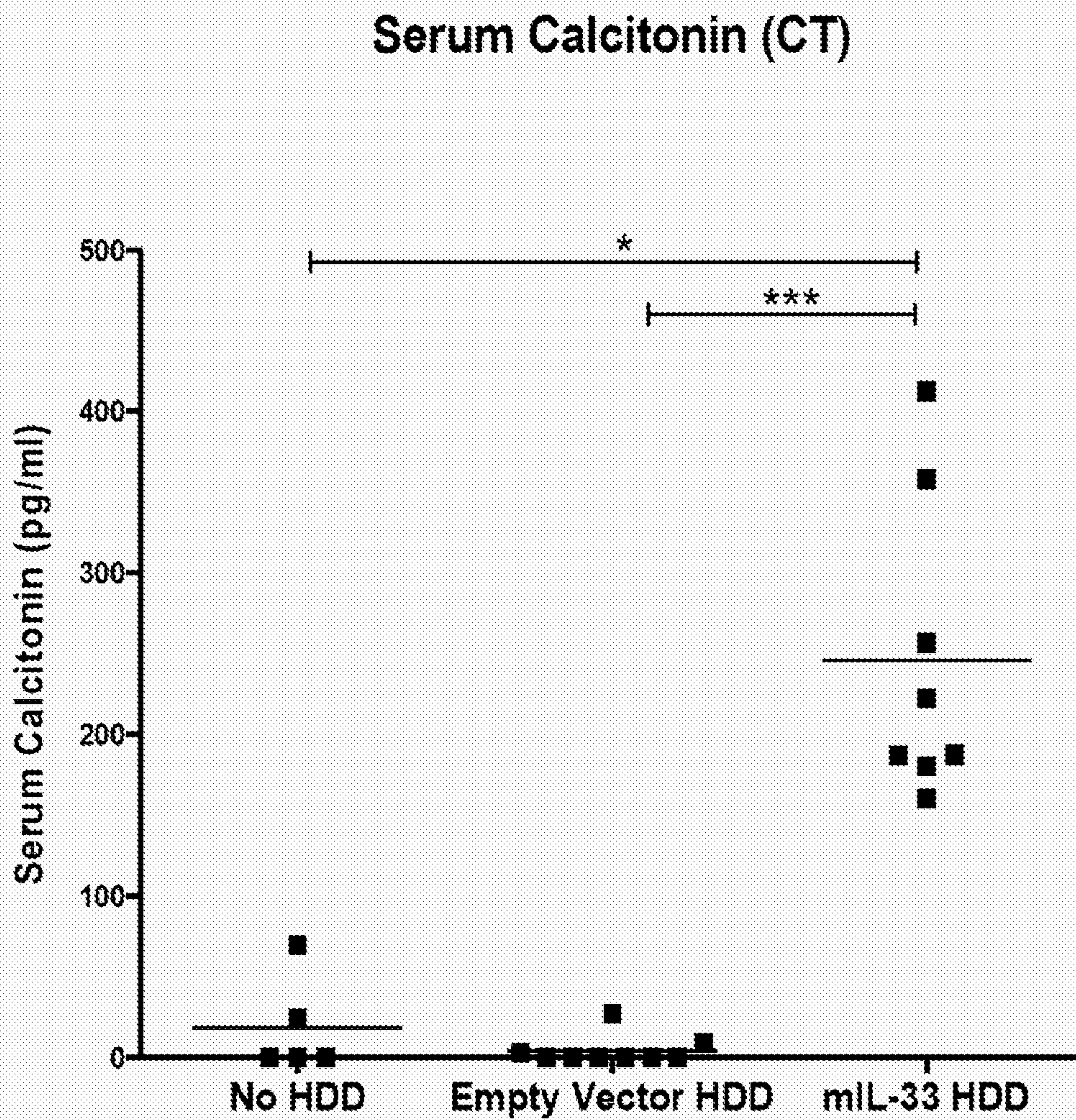
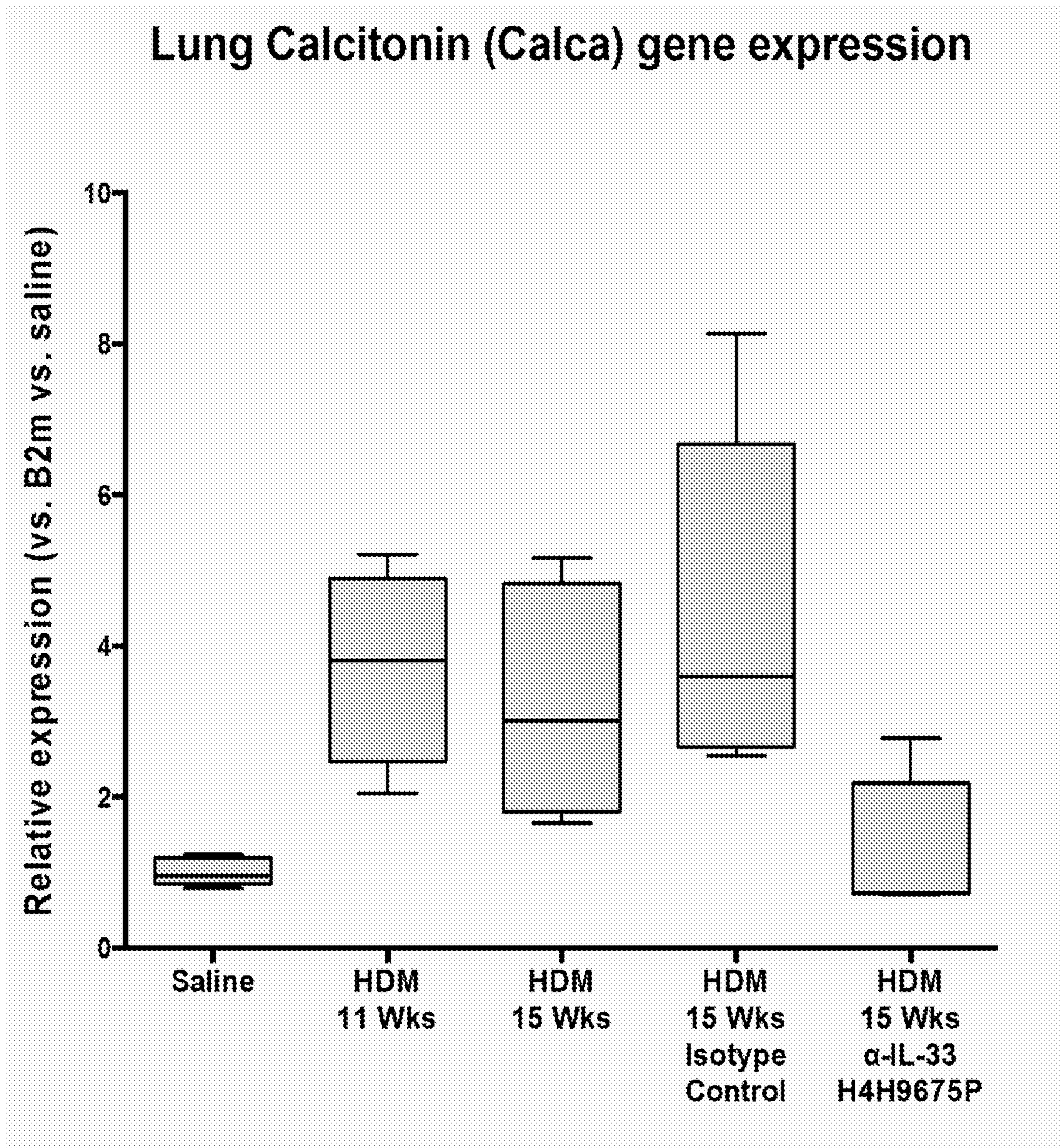
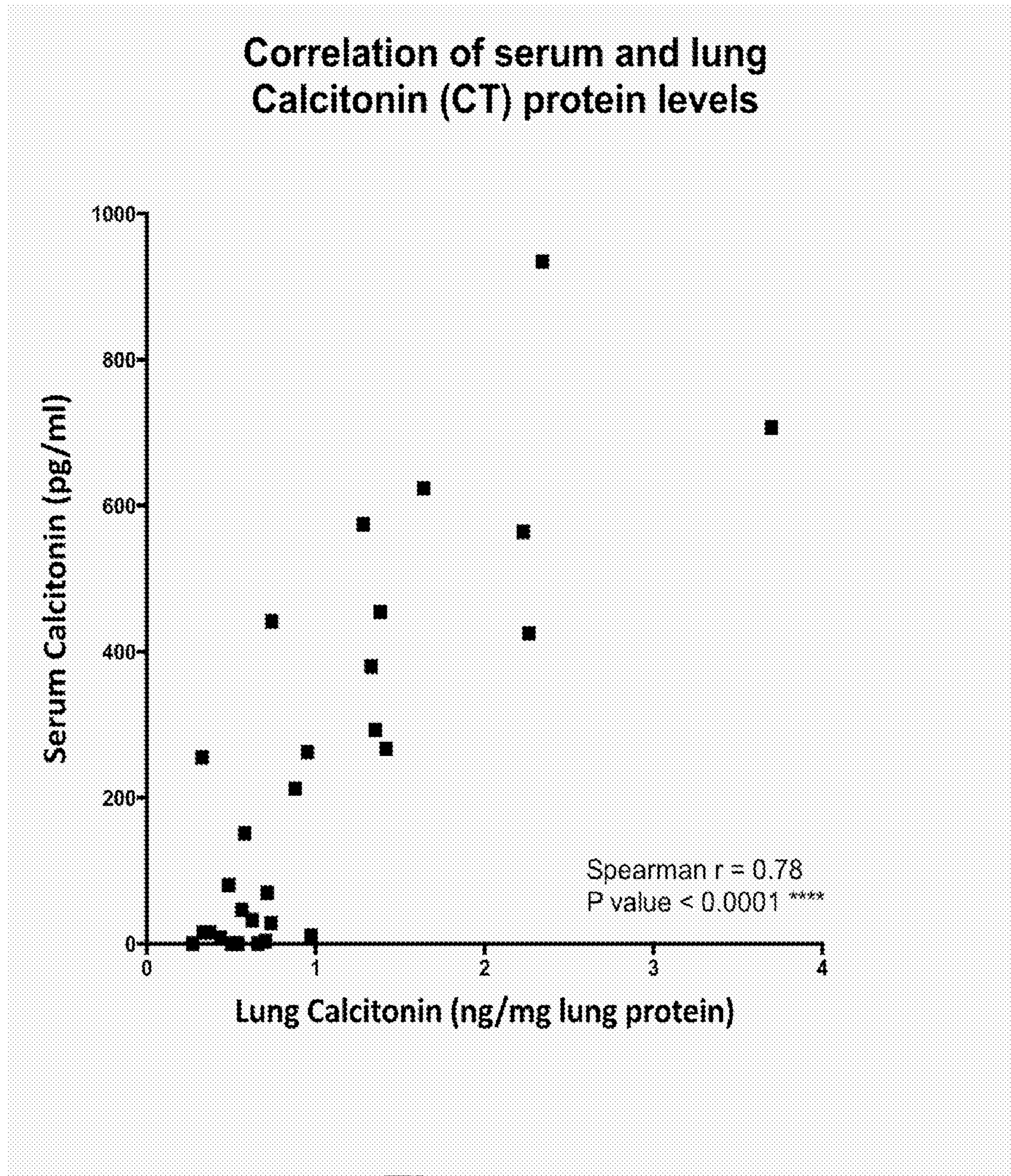
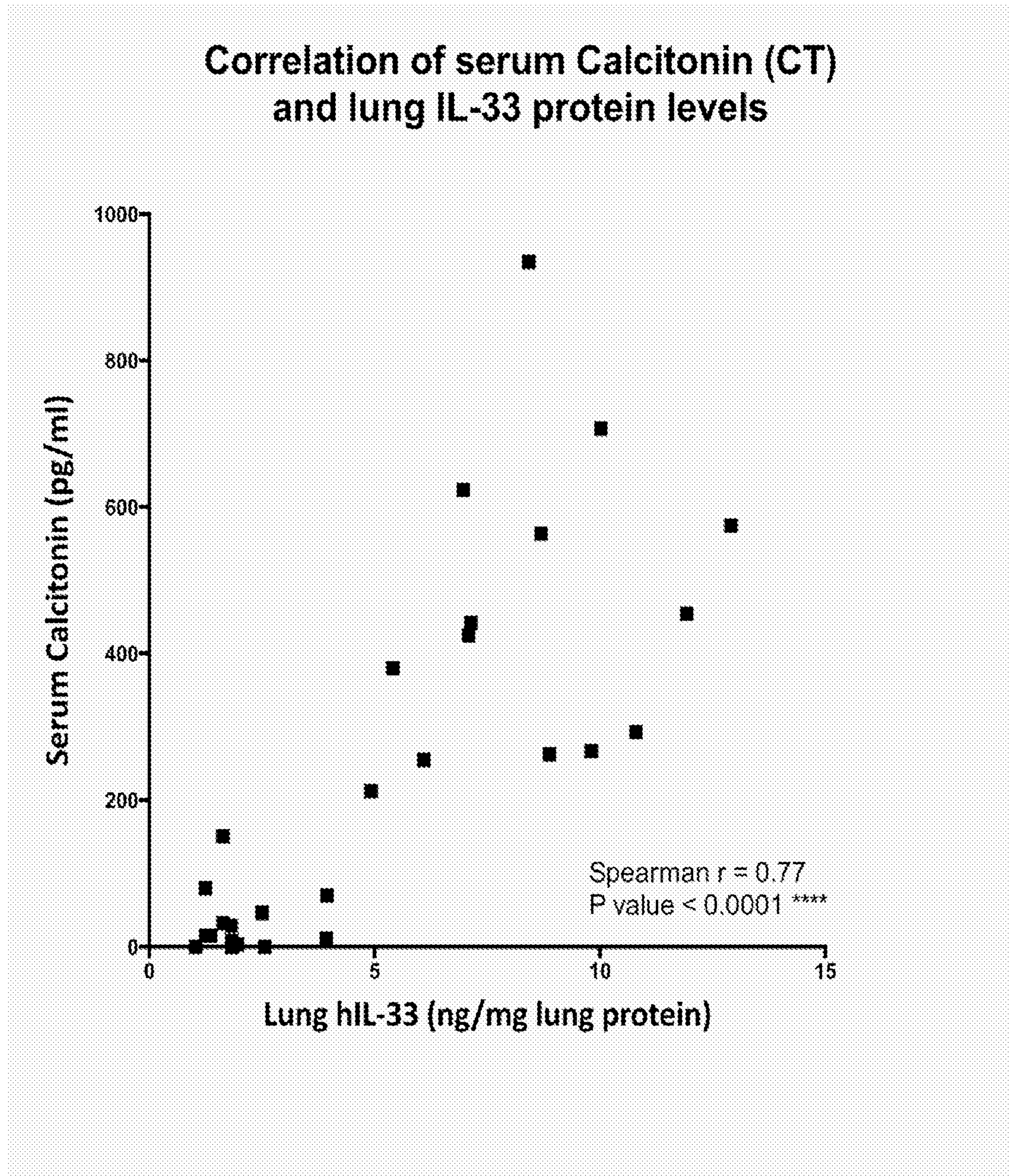


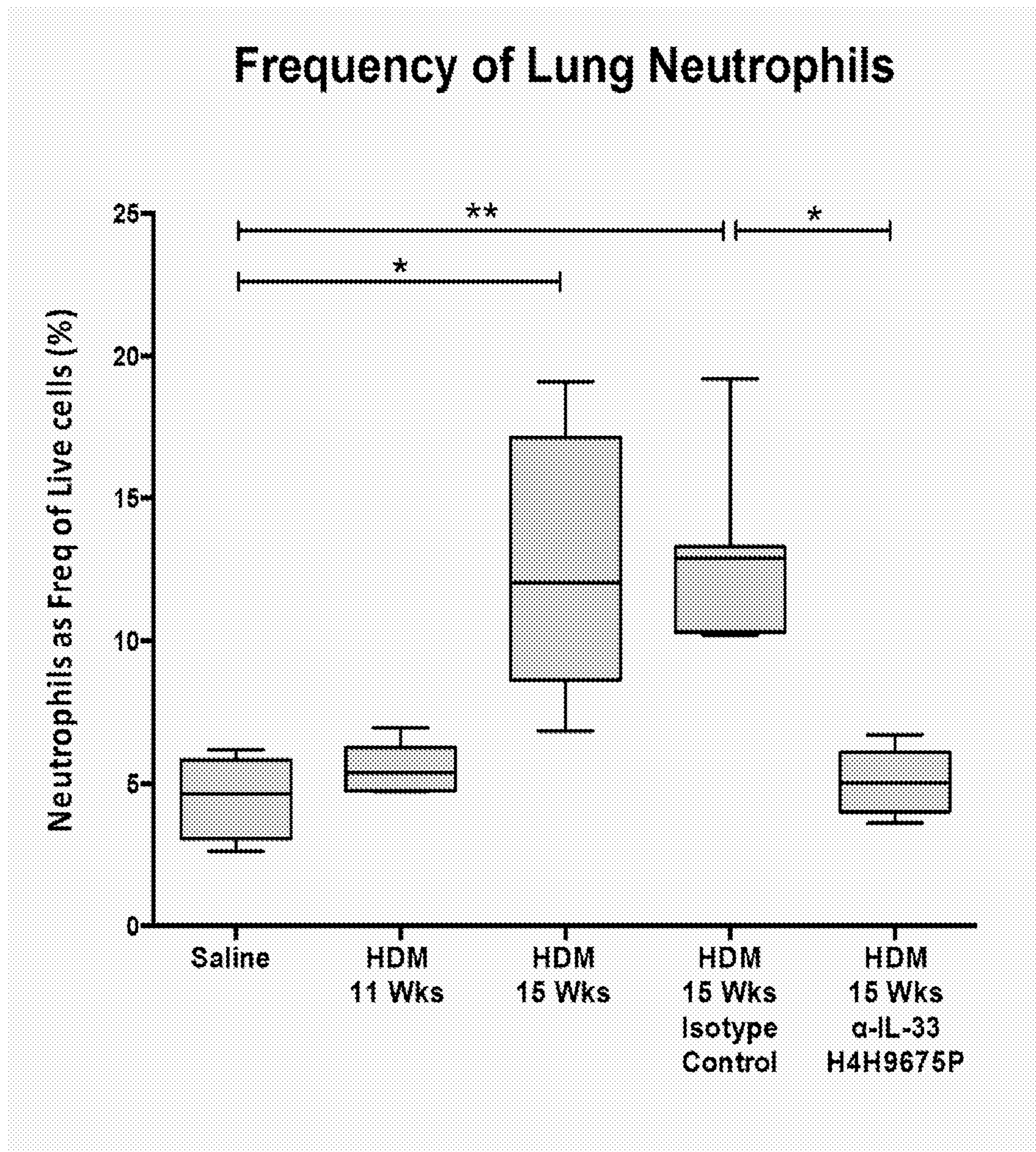
Figure 1B

3/14**Figure 2**

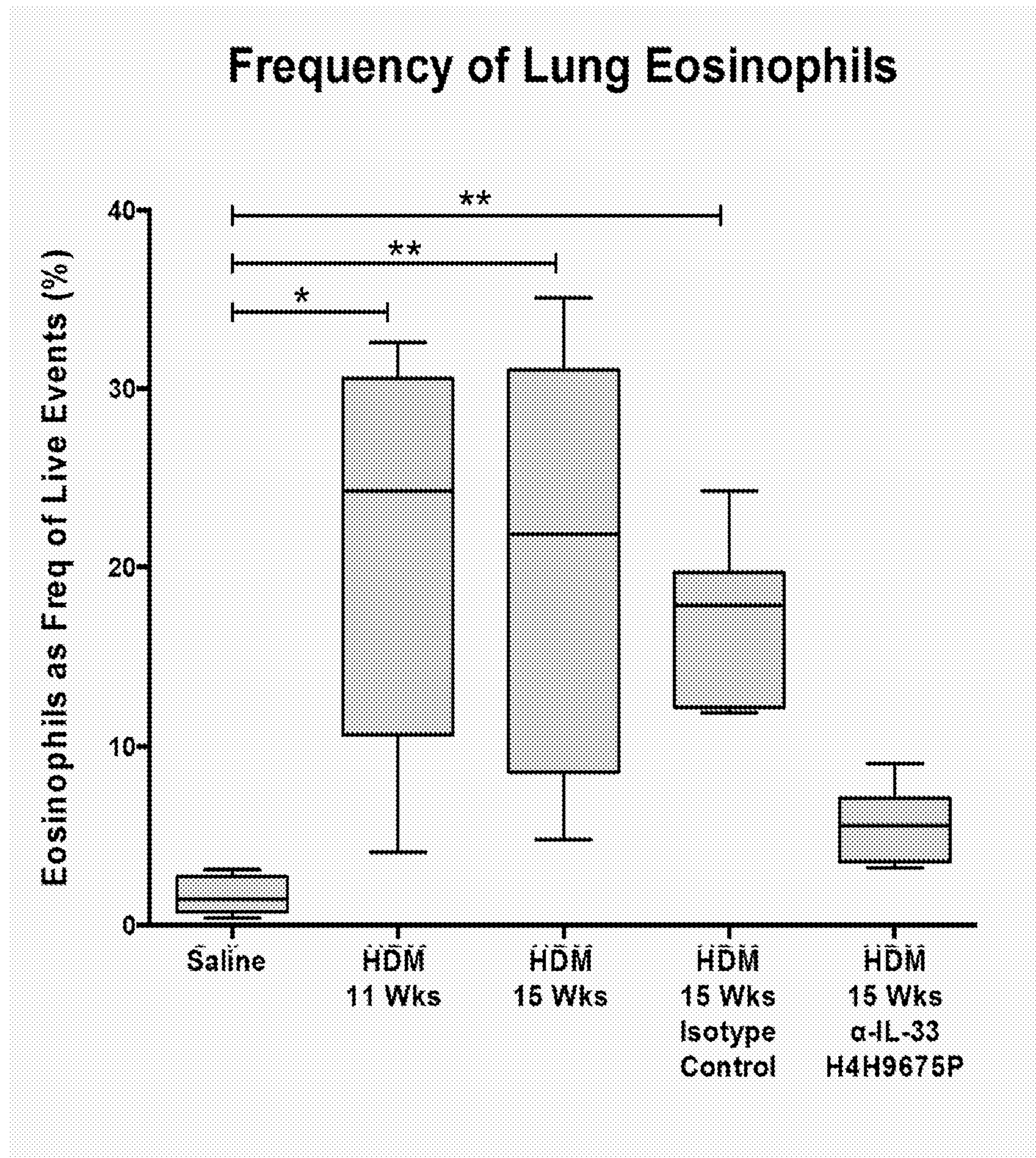
4/14**Figure 3**

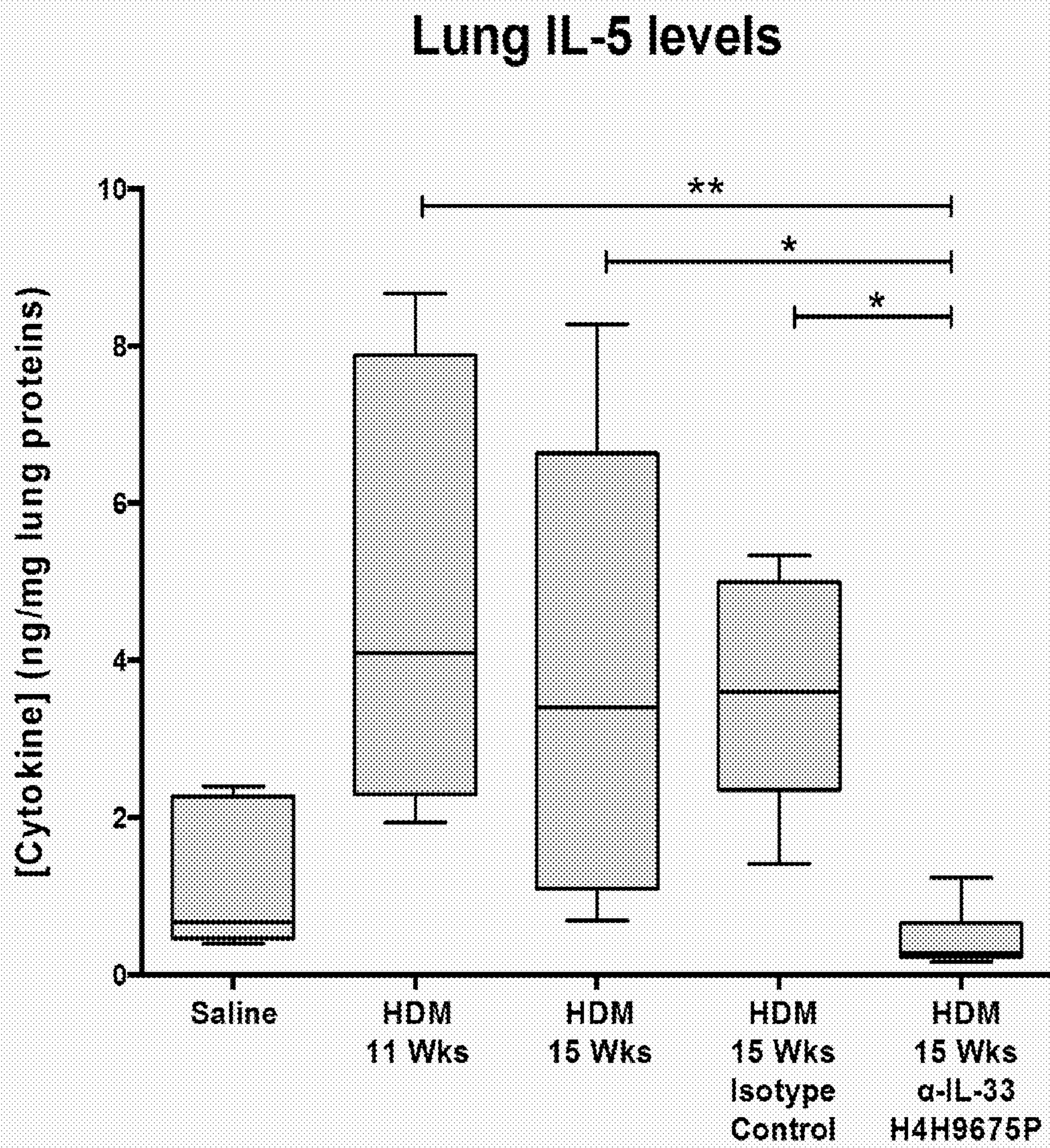
5/14**Figure 4**

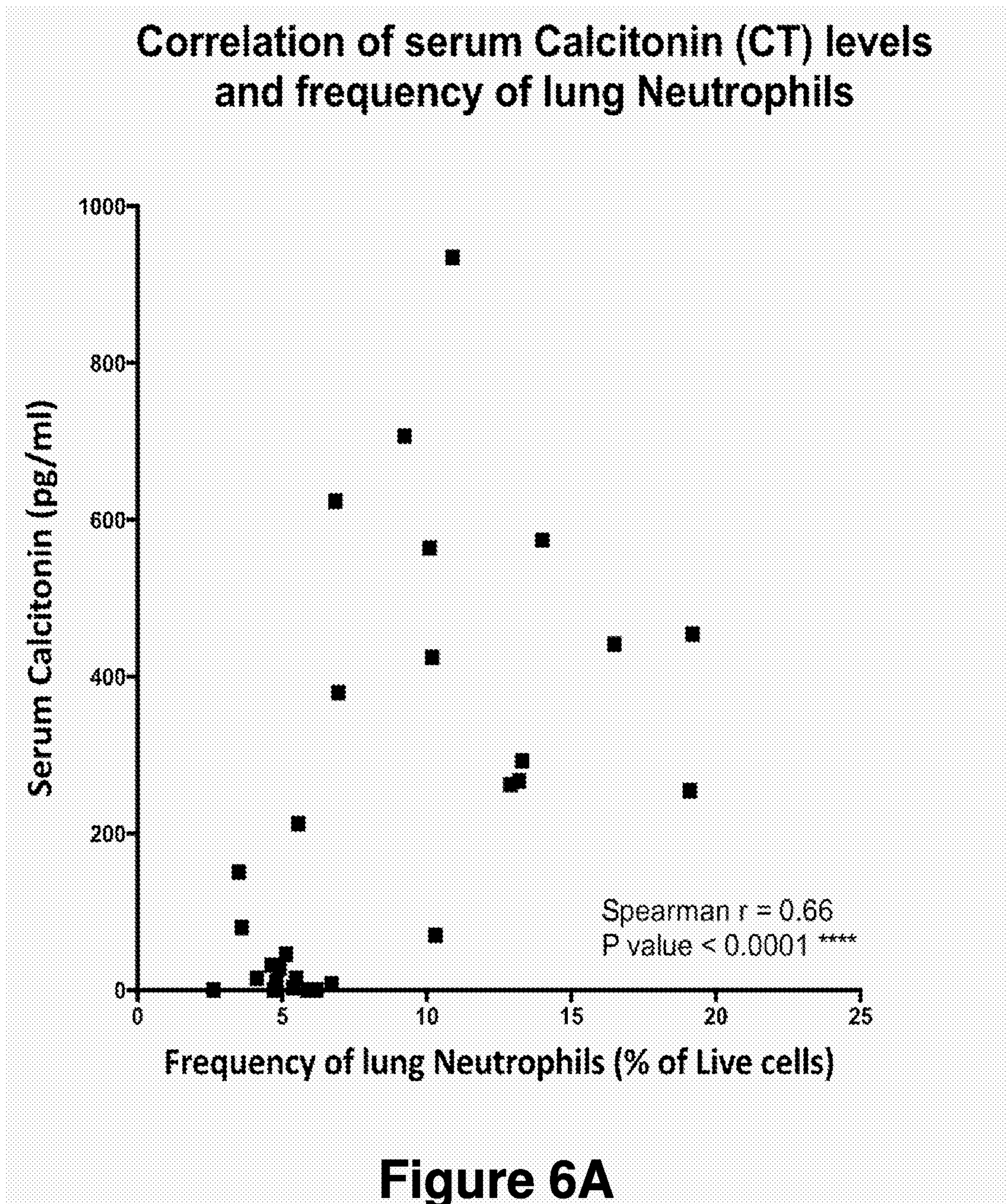
6/14

**Figure 5A**

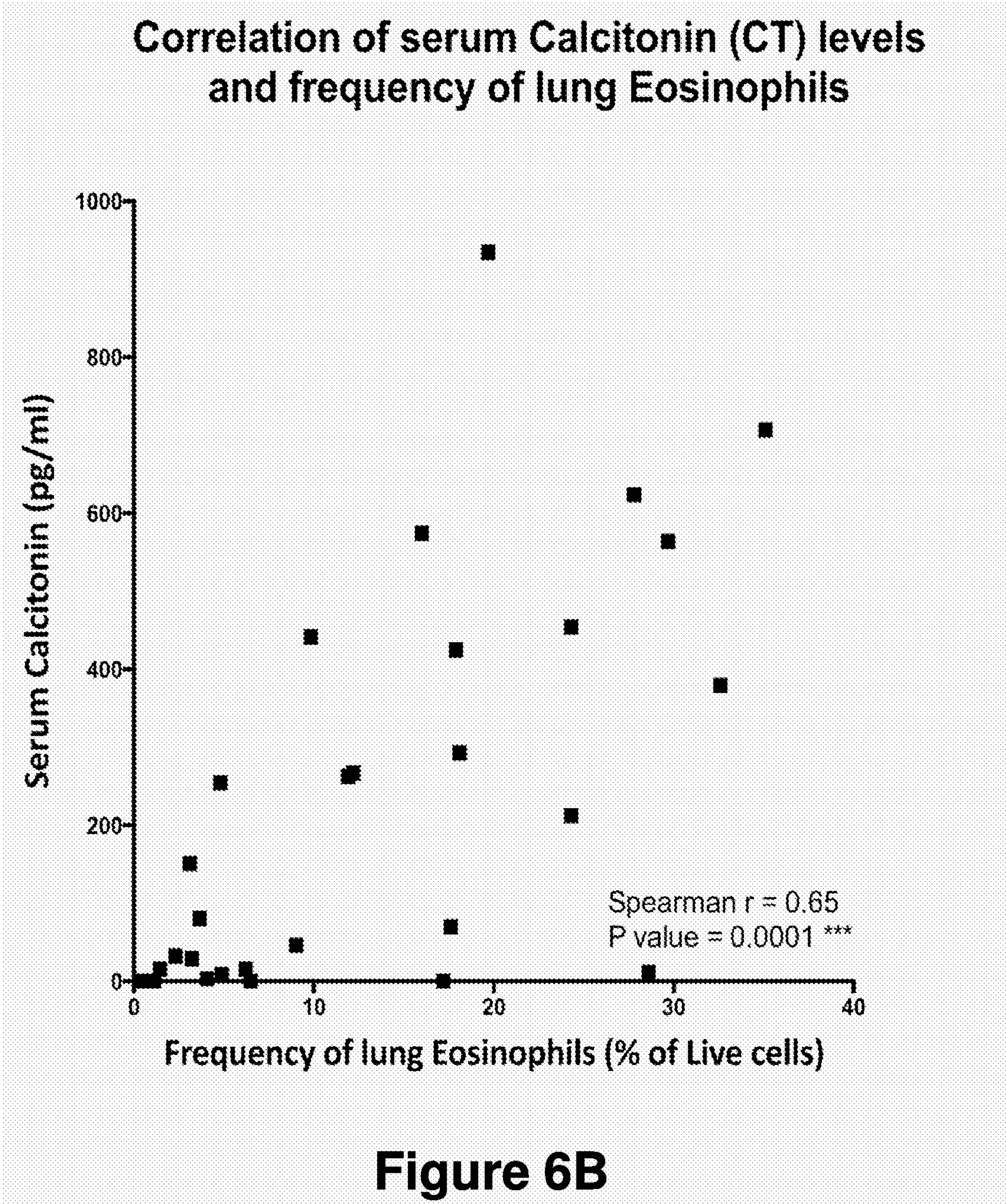
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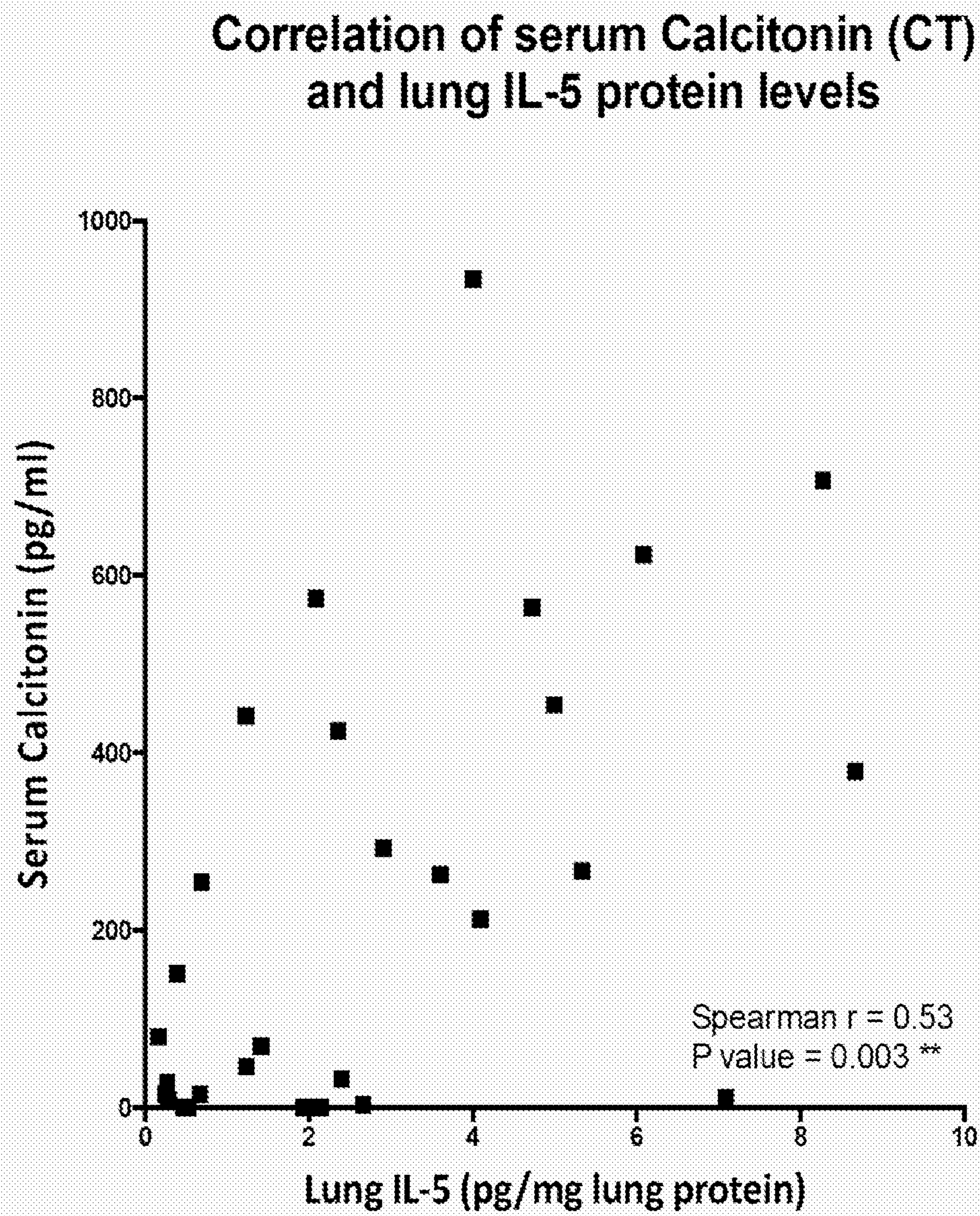
**Figure 5B**

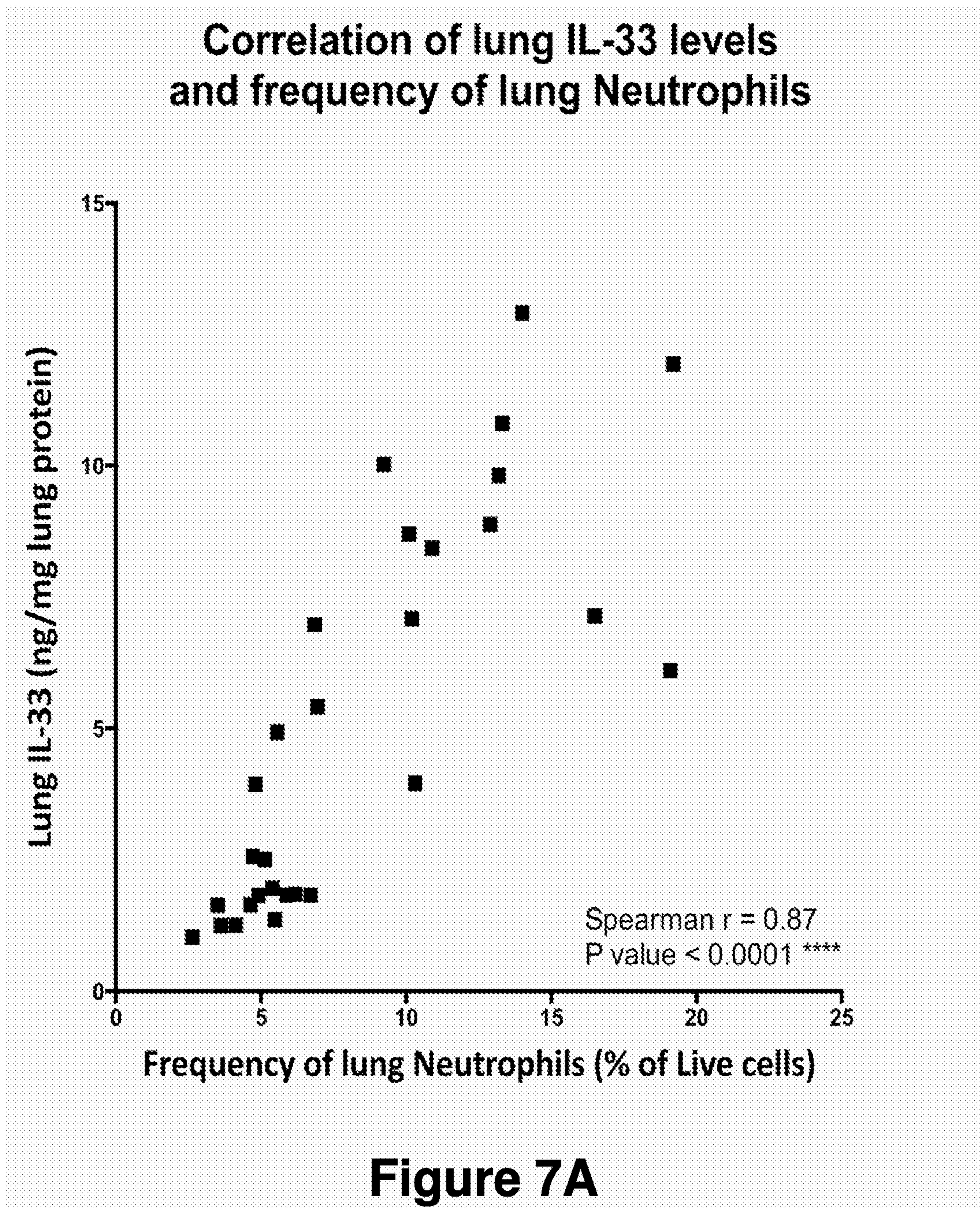
8/14**Figure 5C**

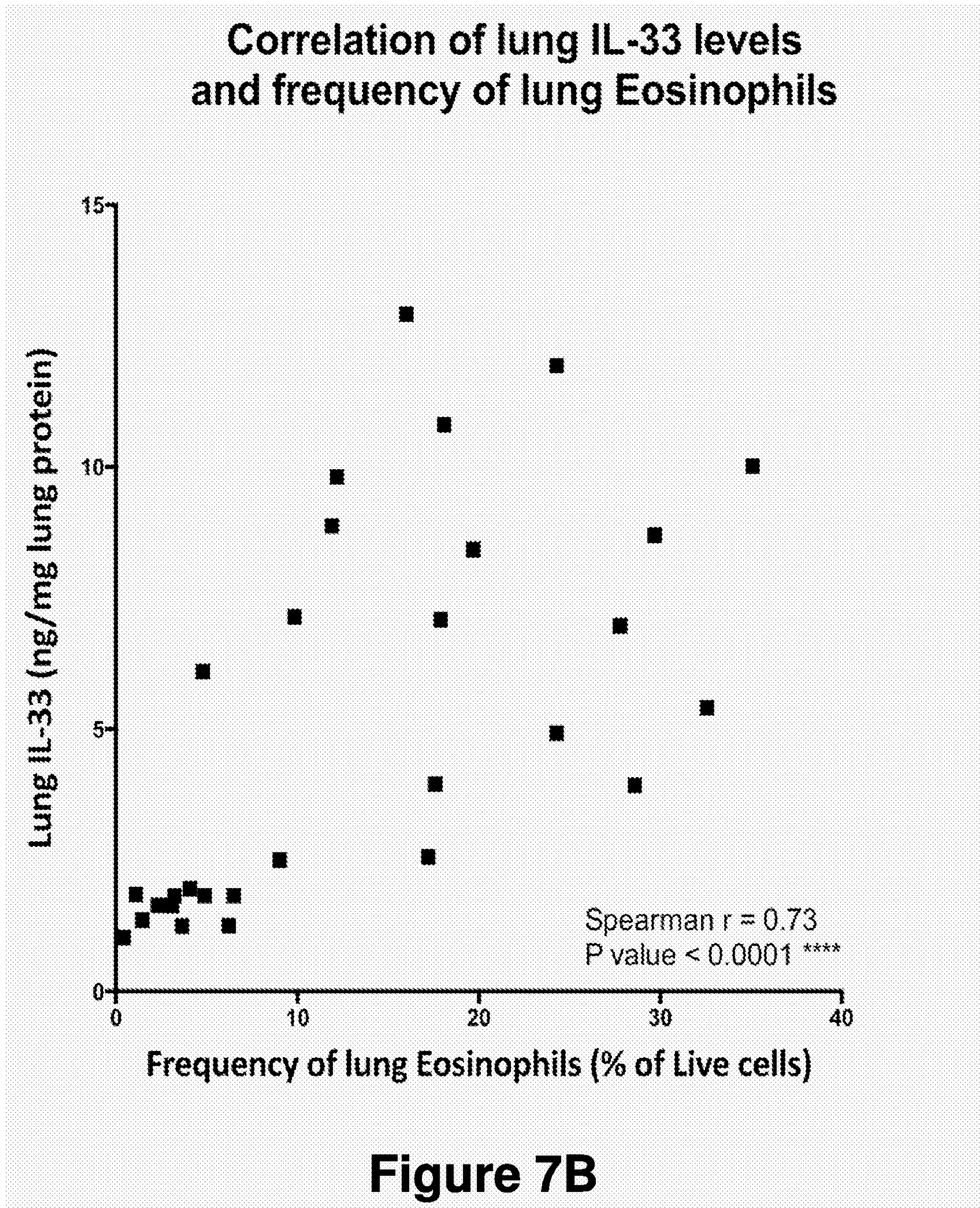
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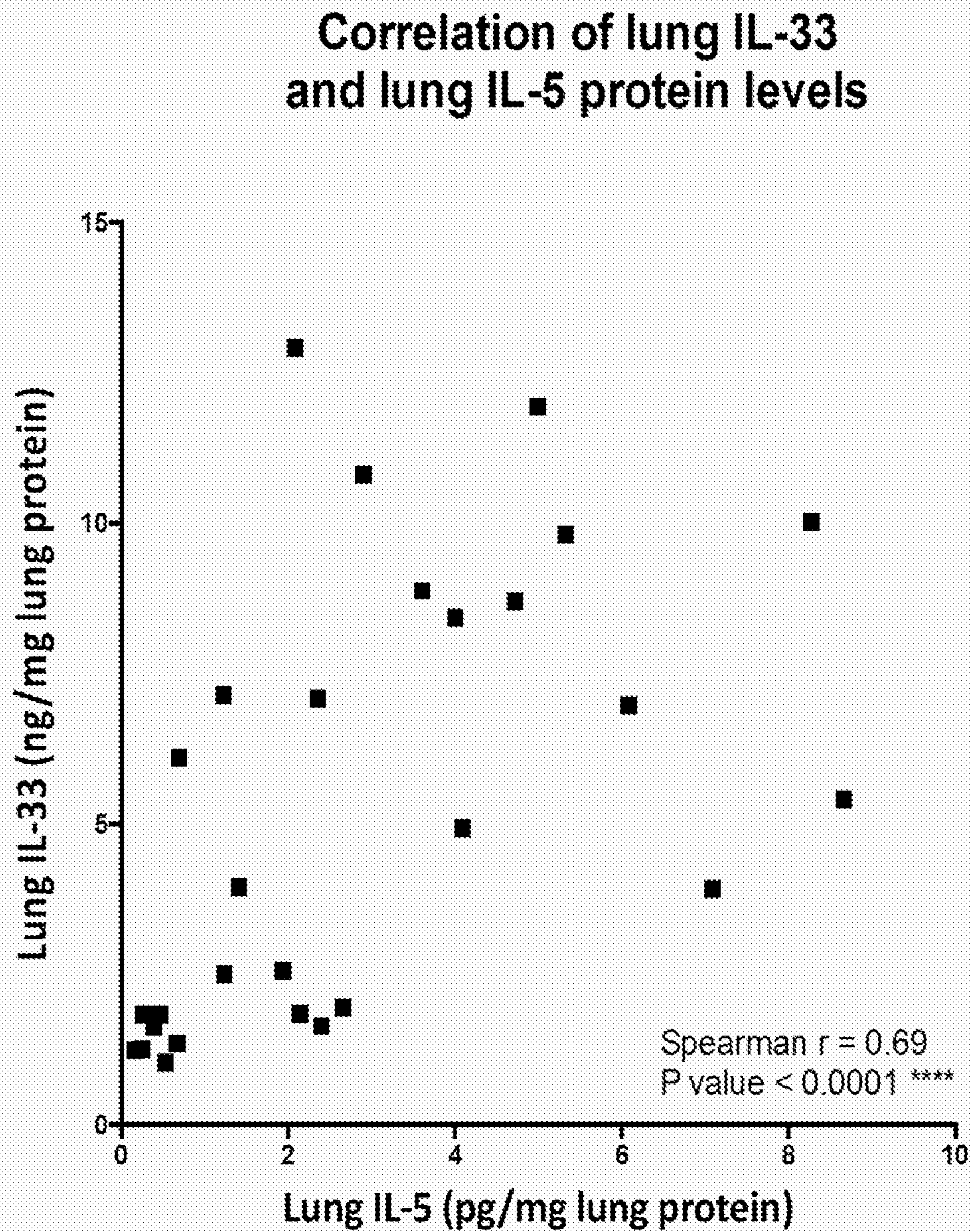
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11/14**Figure 6C**

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14/14**Figure 7C**